RESPIRATORY MEDICINE

A COLLECTION OF CLINICAL PEARLS

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Respiratory Medicine
A collection of clinical pearls

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A Collection of Clinical Pearls
(FIRST EDITION)

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Respiratory medicine is a broadbased specialty that incorporates traditional pulmonary diseases such as infection, airway diseases and cancer in addition to wider aspects such as critical care and sleep disorders. Thus, it may be difficult for the general respiratory specialist to keep up to date with the latest developments across this broad range of diseases and disorders. While there are many textbooks on different aspects of respiratory medicine, there are relatively few that cover the full spectrum of the specialty, and particularly few that have been written by leading experts in all aspects of the discipline.

The present textbook incorporates an extensive series of review papers that have been drawn from review articles published in the Journal of Thoracic Disease over recent years. Typically, these articles have been published in themed issues of the Journal, thus facilitating a comprehensive coverage of each topic. Subjects covered include COPD, sleep disorders, critical care, infections (including tuberculosis), and lung cancer, in addition to broader topics such as the health effects of air pollution. Each chapter is written by leading international experts in the particular topic, and thus provides an authoritative review of the subject concerned. Thus, the book should provide a comprehensive and clinically relevant source of up-to-date information that will interest all general respiratory specialists in clinical practice.

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Remarkable progress has been made in respiratory medicine regarding the diagnostics and therapeutics, paralleled with decades of evolution in this discipline. However, many domains and in-depth mechanisms in the field of respiratory medicine remain unknown, and await our unceasing discovery.

For example, dyspnea, cough, chest pain, hemoptysis, and sputum are five respiratory symptoms frequently seen in many medical disorders. In this sense, a pulmonologist may be frequently encountering with many extrapulmonary diseases. Some patients may concurrently suffer from two or more respiratory diseases, so that pulmonologists ought to take into account all relevant etiological factors in a comprehensive manner, before optimal therapeutic outcomes could finally be reached in the patients. For another example, recent years witness an apparent change in our environment and climate. In particular, the frequently occurring haze has raised global concern. At present, we have verified that during severely hazy days, the hospitalization and clinic visits of patients with chronic obstructive pulmonary disease and asthma are greatly increased. On the other hand, the relationship between atmospheric particulate matters (especially the currently high-level PM2.5 in Asia) and lung cancer is yet to be fully elucidated in long-term observational studies.

In continuing research and practice, global specialists have gained access to a large number of theoretical or observational data about respiratory diseases. These findings are exponentially increasing, and being published in form of papers we read in world-reputed respiratory journals. Given the rapid surge of academic research and hence of article output, it would be of paramount importance to efficiently sort out and put aside relevant articles on the basis of good timeliness and technical pragmatism. Such an effort would enable our pulmonologists, who are heftily engaged in their daily services, to be maximally exposed to and maximally exposed to and enlightened by high-quality studies, and ultimately to improve patient outcomes in the practice.

Journal of Thoracic Disease (*JTD*) is the official publication of Guangzhou Institute of Respiratory Disease (GIRD) and State Key Laboratory of Respiratory Disease. *JTD* keeps abreast with the latest research progress on the prevention, diagnosis and treatment of thoracic diseases, devoting to establish a professional, efficient and open platform for academic communication, and to promote the development of respiratory medicine. Through continued endeavors, we have selected a collection of excellent papers published in *JTD* in the last three years, which are compiled in "Respiratory medicine: A collection of clinical pearls". We are delighted to present the state-of-art papers on topics of interest, including: chronic obstructive pulmonary disease, cough, tuberculosis, interstitial lung disease, lung cancer and others alike. In separate sections, we also have papers on intensive care medicine, sleep medicine and airway intervention. To a certain extent, we hope that our work would have presented the advances in respiratory medicine and clinical techniques in the recent years. It is also noteworthy that the section of "air pollution" in this book deals with potential respiratory problems caused by air pollution, and highlights several updates from studies on this topic. We are optimistic that readers would find this book a reliable and brainstorming friend that would inspire future research on respiratory diseases, and contribute to the daily diagnosis of pulmonologists.

Inevitably, despite our maximal endeavor, there remain pitfalls with regards to the selection, evaluation and editing of articles in this book. We do appreciate readers sharing views and ideas on any aspect of this collection through various channels. Your suggestions and contributions will be reflected in our future editions.

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The fields of respiratory and critical care medicine have grown and changed rapidly during the last couple of decades. From their original definitions, acute respiratory distress syndrome (ARDS) and sepsis have evolved into their new and third versions in 2012 and 2016, respectively, aiming to provide more accurate and rapid diagnoses for patient management. The evolutionary approach of mechanical ventilation using low tidal volume has saved thousands of lives worldwide. There have been many advances in our understanding of several chronic lung diseases from epidemiology and pathogenesis of genetics, inflammation and biomarkers to patient management including pulmonary function testing, pharmacotherapy and non-pharmacological therapy, pulmonary rehabilitation and exercise programmes. Lung cancer immunotherapy by multidisciplinary teams has now emerged as a promising model of precision medicine with several important therapeutic breakthroughs. Since environment has been considered a trigger of lung disease, metabolomics has thus become a rapidly expanding field of systems biology that is receiving significant attention in respiratory research.

While the fields continue to advance rapidly, sufficient knowledge and experience have accumulated to warrant the production of a proceeding from the *Journal of Thoracic Disease* (*JTD*) dedicated to respiratory and critical care medicine.

Modern respiratory medicine involves many disciplines that are represented by, not only classic organ-based subspecialties of medicine, but also the specialties of critical care, surgery and anaesthesiology. In this first edition, we chose to limit the subject material of our proceeding to "respiratory medicine" in order to allow for the production of a highly readable book that can serve equally well for comprehensive review and as a reference source. We have tried to extract what seems to have made significant advances to our knowledge that have shaped and may future have potential impact on our own approach to daily practice in the field.

This book is envisioned to include multiple authors of a truly exceptional group of clinician-scientists actively involved in the field and is targeted primarily at practicing physicians who spend a large part of their time caring for patients at bedside. Each chapter of this book consists of a comprehensive review of pertinent clinical diagnosis, pathophysiology data and management issues. Substantial references are provided for readers to explore subjects in greater detail; thus, the book is appropriate for respirologists as well as intensivists, surgical or anesthesia specialists. The goal is to update the acknowledged excellence of best practice in respiratory medicine.

We wish to thank the extremely dedicated editorial staff that provided us with outstanding organizational and editorial assistance to complete a venture of this magnitude. A special thank goes to Professor Guangqiao Zeng, the editorial director of *JTD* and editorial moderator of this book, who organized and oversaw the editorial process of *JTD* and the proceeding series.

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Importance of airway geometry and respiratory parameters variability for particle deposition in the human respiratory tract

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The quantitative estimation of particle deposition in the respiratory tract is the important practical step for prediction of potential health outcome of inhaled aerosols. Such knowledge is indispensable in assessing aerosol toxicology as well as in the optimization of the drug delivery to the lungs by inhalation (1-3).

This problem is complex since many factors simultaneously play a role in this process, resulting in a high variability of regional and total deposition efficiency. These most important factors can be listed as follows:

a) the respiratory tract geometry, which is highly variable with age or body size and health   conditions

b) the breathing pattern, which depends on the age and health, but also on the momentary physical activity

c) aerosol properties (particle size, shape, density, hygroscopity, surface properties, etc).

Aerosol particles, depending on their size and mass, can be deposited in different parts of the respiratory system due to action of several physical mechanisms, where the inertial impaction, gravitational settling and Brownian diffusion are the predominant ones.

Several approaches are used to estimate quantitatively the aerosol deposition fractions in different regions of the respiratory tract. The recent concepts - which are still under development - employ the Computational Fluid Dynamics (CFD) to identify the air flow pattern and motion of aerosol particles in the human respiratory system (4). This technique is demanding both from conceptual and computer-power viewpoints. Providing that the regional geometry of the air passages can be implemented correctly in form of the numerical mesh, CFD allows for a very detailed flow analysis of inhaled aerosol particles in the network of the real airways. Any type of physiological situation can be potentially modeled with this computational approach, including diseased lungs, pediatric patients, etc. Anyway, the methodology is not straightforward and quite often simplifying assumptions are introduced into the models to obtain the results in the reasonable computational time and accuracy. For instance, CFD simulations for the respiratory system are typically simplified by assuming constant airflow rates (5-9), although such presumption is far from the reality. Recently, the more accurate simulations for the unsteady airflow patterns (i.e. the real-like breathing curves) has become available (10-12).

Another method used for the estimation of aerosol deposition in the human respiratory system employs semi-empirical models which were developed taking into account the averaged deposition data obtained *in vivo* and *in vitro* (13-15). In this method the information on the deposition in the whole lungs as well as in the selected regions of the respiratory system can be analyzed. The advantage of such approach is its mathematical lucidity, although the proposed equations certainly are lacking the universality as they do not address many of the significant factors indicated earlier.

In any method of quantitative prediction of aerosol deposition the important problem is their validation due to the large scatter of *in vivo* data (variability) observed among different subjects (16). In this issue of the Journal of Thoracic Disease, Hussain *et al.* discuss the problem of variability in the estimated regional deposition fractions of inhaled aerosol particles, which is caused by intersubject

differences of extrathoracic (ET) dimensions and breathing patterns (17). The authors use selected models (13,14,18-20) to calculate the deposition efficiency in nasal and oral regions for variable airways dimensions taken from the literature (21,22), and then combine these results with the stochastic IDEAL model (23,24) which allows to determine the deposition fractions in each generation of the tracheobronchial tree. In effect, the authors are able to demonstrate that variability in ET geometry have a noticeable influence on the total and regional deposition fraction, both for oral and nasal breathing. Obviously, such deposition data are dependent on breathing regime, what is also demonstrated in the paper (breathing at rest *vs.* light exercise). Based on their computational results, the authors show that intersubject variability of the ET geometry is an important factor for the regional and total aerosol particle deposition in the respiratory tract. It is also concluded that some more general metrics of ET anatomy (e.g., the scaling factor: SF) can be appropriate for description of the intersubject deposition variability.

This interesting paper does not cover of course the full complexity of the problem which was stated in the beginning of this editorial. However, the presented results - according to the authors' conclusion - may serve as a baseline to analyze more complicated cases in the future, e.g., for the lung pathology, when the intersubject deposition variability is expected to be much higher (the reasons for that are the different regional geometry of diseased lungs due to airways obstructions and highly variable airflow patterns - for some recent data on that issue see for example (25).

Readers of the paper by Hussain *et al.* will also take an additional advantage from the comprehensive presentation of the most common semi-empirical computational models that can be used for the estimation of aerosol deposition in the human respiratory system. This undoubtly helps to recognize the importance of different physical mechanisms in the deposition of inhaled aerosol particles with different sizes, but also of the parametric sensitivity of the process under consideration. The awareness in this aspect seems to be essential in the designing the effective therapy of lung diseases by inhalation of aerosolized medicines.

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Pneumonia and empyema: causal, casual or unknown

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Abstract: Parapneumonic effusions complicating pneumonia are associated with increased morbidity and mortality. Along with increased mortality, complicated parapneumonic effusion and empyema often necessitate prolonged treatment, longer hospital stay and interventions. Parapneumonic effusions arise from inflammation in the lungs and pleural space from direct invasion of bacteria, cascade of inflammatory events and bacteriologic virulence features. Patient factors and comorbidities also contribute to the pathophysiology of parapneumonic effusion development. The evolution of parapneumonic effusion can be divided into three progressive stages: (I) exudative stage; (II) fibrinopurulent stage; and (III) organizing stage with pleural peel formation. These stages can help categorize effusions into groups in order to evaluate the risk of a complicated course requiring intervention. We recommend that clinical data be evaluated and a stepwise approach be taken in management of these patients. This review article discusses current understanding of the development and relationship of parapneumonic effusions with pneumonia.

Keywords: Parapneumonic effusion; pleural infection; empyema; pneumonia

Community acquired pneumonia results in significant morbidity and mortality, and is the 9th leading cause of death in the United States when combined with influenza (1). About 6 million cases are reported annually in the United States population, resulting in 500,000 to 1.1 million hospitalizations annually (2,3). Among pneumonia patients admitted to the hospital, 20% to 40% have pleural effusion, and 10% of these develop complicated parapneumonic effusion or empyema (4,5). There is considerable variation in the course and aggressiveness of parapneumonic effusions; therefore an understanding of its progression is important. Along with increased mortality, complicated parapneumonic effusion and empyema often necessitate prolonged treatment, longer hospital stay and interventions. Thus, identification of these patients and prompt management is critical.

This review article discusses current understanding of the development and relationship of parapneumonic effusions with pneumonia.

Historical information

An Egyptian physician, Imhotep was most likely the first to describe pleural infections around 3000 BC, however Hippocrates is more often cited for its recognition in 500 BC. It was not until the 19th century that open lung drainage was recommended for treatment, however mortality was as high as 70%, likely related to complications of surgery. Closed chest tube drainage was first described in 1876 and widely used during the influenza epidemic of 1917-1919, resulting in improved survival (6,7). The introduction of antibiotics has not only reduced the incidence of empyema, but also changed its bacteriology. In the pre-antibiotic era, 60-70% of empyemas were due to *Streptococcus pneumoniae* (*S. pneumoniae*), which now only accounts for about 10% (8-10). *Staphylococcus aureus* empyema has become more common, along with anaerobic and Gram-negative bacteria infections (7,8,10-14). With the advent of antibiotics, the incidence of empyema was dramatically reduced; however

more recent studies indicate the incidence of pleural infection is increasing (15,16).

Parapneumonic effusion is defined as any pleural effusion secondary to viral or bacterial pneumonia or lung abscess. "Complicated" parapneumonic effusion is a paraneumonic effusion that requires an invasive procedure, such as tube thoracostomy, to resolve, often with positive pleural fluid cultures (5). Empyema is defined by the presence of bacteria or pus in the pleural space. Pus is thick, viscous fluid that appears purulent. About 60% of empyemas are related to a primary pneumonic process, therefore risk factors for pleural infection are similar to those for pneumonia (3,17). However, up to 40% of empyema may be secondary to a non-pneumonic process, such as systemic infection with hematogenous spread or abdominal etiology. Independent risk factors for the development of empyema include diabetes, immunosuppression, gastro-esophageal reflux disease, alcohol and intravenous drug abuse, aspiration, and poor oral hygiene (18). Other causes of empyema include complications after thoracic surgical procedures, trauma, esophageal perforation, thoracentesis and subdiaphragmatic infection (3,17).

Pathophysiologic features

In homeostatic conditions, pleural fluid arises from the systemic pleural vessels, traverses across leaky pleural membranes into the pleural space and exits via the parietal pleural lymphatics in the dependent part of the cavity (19,20). In healthy adults, the pleural space contains a small volume (1-20 mL) of low protein fluid that forms a lubricating film about 10 μm thick between the visceral and parietal pleural surfaces (6,19). A pressure gradient facilitates movement into, but not out of the pleural space, as intrapleural pressure is lower than interstitial pressure, and pleural membranes are leaky, offering little resistance to liquid or protein movement. The majority of pleural fluid exits the space by bulk flow, rather than diffusion or active transport, through the parietal lymphatics (20). Pleural fluid turnover is estimated to be ~0.15 mL/kg·h (19).

Pleural fluid accumulates when the rate of formation exceeds the rate of absorption. The flow of pleural lymphatics can efficiently increase in response to an increase in pleural fluid filtration, acting as a negative feedback mechanism. The lymphatic flow is about 15 mL/day, as this is the typical amount of pleural fluid formed per day. However, the capacity of the lymphatics is about 300-700 mL/day. Due to the large lymphatic capacity, unless the lymphatic drainage is severely impaired, another factor must be present for pleural fluid to accumulate (3,19).

The most common cause of increased pleural fluid formation is increased interstitial edema. This can occur as a result of several processes and is the predominant mechanism for the formation of parapneumonic effusions along with pleural effusions related to congestive heart failure (CHF), pulmonary embolism and acute respiratory distress syndrome. Decreased pleural pressures can also contribute to pleural fluid accumulation, as in advanced empyema when the visceral pleura becomes coated with a collagenous peel and traps the lung. Increased capillary permeability, particularly when the pleura becomes inflamed, also contributes to pleural effusion formation. Lymphatic obstruction is a common mechanism contributing to malignant effusions (3,19).

The evolution of parapneumonic effusion is divided into three progressive stages: (I) exudative stage; (II) fibrinopurulent stage; and (III) organizing stage with pleural peel formation (21). In the early exudative stage there is a rapid outpouring of fluid and inflammatory cells into the pleural space due to increased capillary microvascular permeability. This directly results from proinflammatory cytokines, such as interleukin 8 (IL-8) and tumor necrosis factor α (TNF-α) (22,23). The inflammatory process of the pulmonary parenchyma extends to the visceral pleura causing changes to the mesothelial cells lining the pleura, allowing increased fluid movement. This causes a local pleuritic reaction, and the characteristic pleuritic chest pain described by patients (5,21). Researchers studying rabbits infected with intrapulmonary *Pseudomonas* found a dose dependent relationship between bacterial levels and extent of alveolar epithelial injury, which further facilitated entry of alveolar protein and bacteria into the pleural space. This occurred within hours of inoculation (24). See *Figure 1*.

The pleural fluid in this early exudative stage is usually clear free-flowing exudative fluid with predominance of neutrophils, and characterized by negative bacterial cultures, glucose level greater than 60 mg/dL, pH above 7.20, lactic acid dehydrogenase (LDH) less than three times the upper limit of normal for serum (often <1,000 units/L) and low white cell count (4,5,25-31). Pleural fluid that develops during this stage is usually considered a "simple" parapneumonic effusion and treatment with antibiotics is often adequate, without the need for tube drainage (4,27,28).

Patients can progress to stage 2, the fibrinopurulent stage within hours if effective treatment is not provided. This next stage is characterized by deposition of fibrin clots and
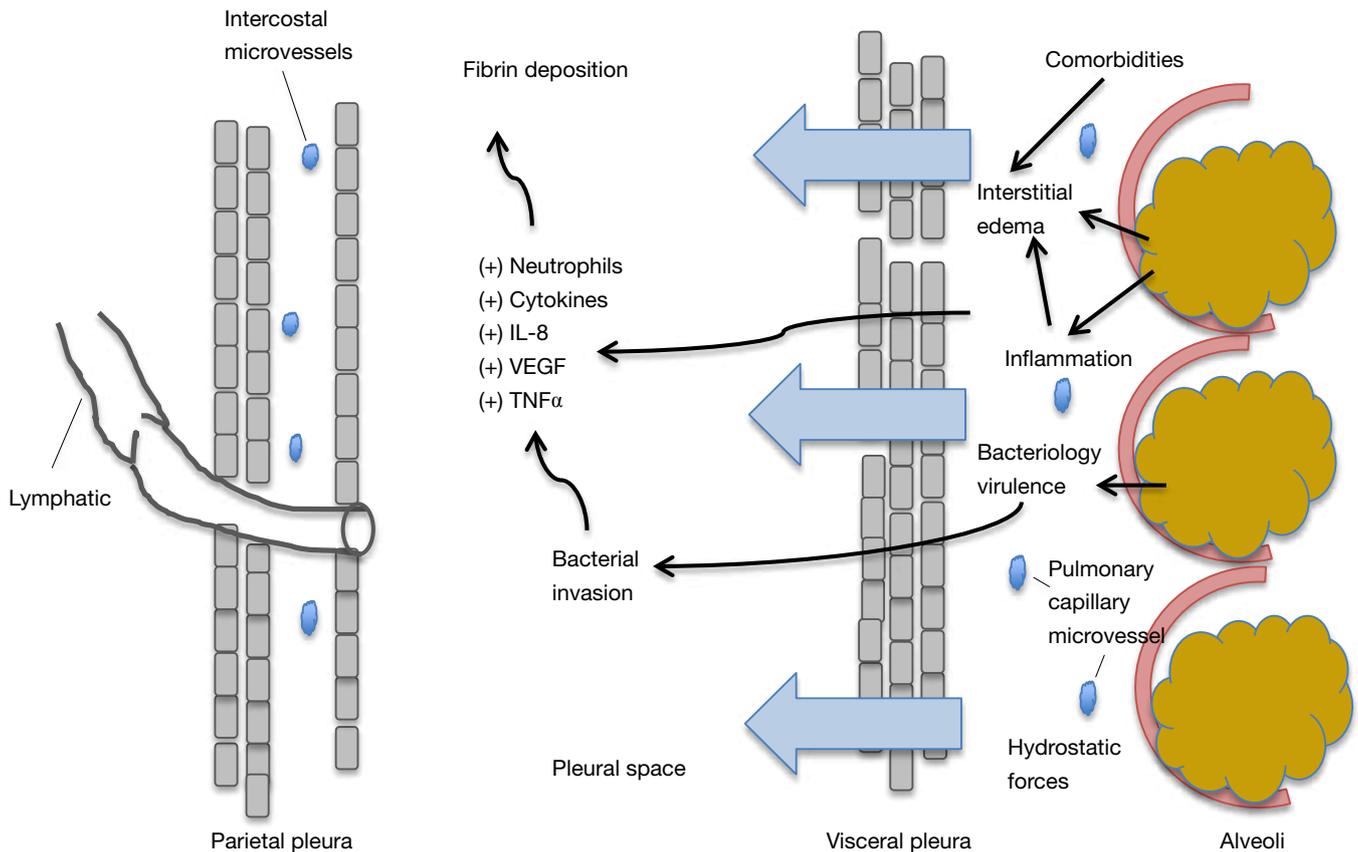
Figure 1 Schema shows mechanism of pleural effusion development in pneumonia. Initial bacterial infection causes local inflammatory reaction resulting in increased capillary microvascular permeability and a rapid outpouring of fluid containing inflammatory cells into the pleural space. Comorbidities such as heart failure also further contribute to interstitial edema. IL-8, interleukin 8; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

fibrin membranes in the pleural space, leading to loculations and isolated collections of fluid. Invasion of bacteria from the pulmonary parenchyma occurs across the damaged endothelium. This invasion accelerates the immune response and directly contributes to fluid loculation (25) by promoting further migration of neutrophils and activation of the coagulation cascade. This leads to increased pro-coagulant and decreased fibrinolytic activity, which encourages fibrin deposition and promotes formation of septations within the fluid. The inflammatory reaction is further fueled by neutrophil phagocytosis and bacterial death, which results in release of more bacteria cell wall derived fragments and proteases (22).

This process has been demonstrated in mice with empyema after being infected intranasally with *S. pneumoniae*. Researchers found rapid bacterial invasion and increased inflammatory markers in the pleural space, such as

IL-8, vascular endothelial growth factor (VEGF), monocyte chemotactic protein 1 (MCP-1) and TNF-α, which caused significant neutrophilia and development of fibrinous pleural adhesions. The pleural cavity offered a protected compartment for the bacteria, as bacterial clearance from the pleural space was poor in this animal model (31).

The pleural fluid in the fibrinopurulent stage is often turbid and characterized by positive bacteria on Gram stain and culture. Cytology shows neutrophils and degenerated cells. The combination of bacterial invasion and increased inflammatory response leads to increased lactic acid and carbon dioxide production, resulting in a fall in pleural fluid pH, increased glucose metabolism and a rise in LDH, consistent with "complicated" parapneumonic effusion. Typical pleural fluid studies in this stage have a glucose level less than 60 mg/dL, pH below 7.20, and pleural LDH more than three times the upper limit normal for serum

Table 1 Parapneumonic effusion classification				
Anatomy	Bacteriology	Chemistry	Category	Drainage intervention
Very small to small free flowing effusion*	Unknown	Unknown	1	No
Small to moderate free flowing effusion*	Negative culture and Gram stain	Normal pH and glucose	2	No
Large effusion or loculation*	Positive culture or Gram stain	Low pH or glucose	3	Yes
Any size	Pus		4	Yes
*, small <10 mm on lateral decubitus; moderate less than half hemithorax; large greater than or equal to half hemithorax. Risk of poor outcome is very low to low for category 1 and 2; however category 3 and 4 have moderate to high risk.				

(often >1,000 units/L) (5,25).

If stage 2 pleural fluid is not drained in conjunction with effective antibiotic therapy, the effusion may progress to stage 3, the organizing stage. This final stage is characterized by fibroblasts that proliferate and invade the pleural fluid from both the visceral and parietal pleura, forming a thick pleural peel. Fibrin membranes are transformed by fibroblast into a web of thick nonelastic pleura. This can occasionally encase the lung, preventing re-expansion and resulting in "trapped lung". This can functionally result in impaired gas exchange and produce a persistent pleural space, increasing risk for continued infection (6,25). Clinical course varies considerably, from spontaneous healing with persistent defects of lung function, to chronic forms of empyema with high risk of complications, such as bronchopleural fistula, trapped restricted lung, fibro-thorax or spontaneous perforation through the chest wall (25).

Classification

Classifying the prognosis of a patient with a parapneumonic effusion is a critical first step in management. In 2000, the American College of Chest Physicians developed a classification system based on the anatomic features (A), bacteriology (B), and chemistry of the pleural fluid (C) (32). The anatomy (A) of the pleural fluid is based on three features; size, whether it is free flowing and whether the parietal pleura are thickened. The bacteriology (B) of the effusion is based on whether pleural cultures or smears are positive. The chemistry (C) of the pleural fluid is based on pH measured with a blood gas machine. Pleural fluid glucose can be used as an alternative to pH with a cutoff level of 60 mg/dL. Based on the A, B, and C classification, the effusion is categorized. Risk of poor outcome is based on the category of the effusion, as are recommendations to drain the effusion (*Table 1*) (5,32). Similarly, the British

Thoracic Society has published a diagnostic algorithm for management of these patients (6).

Bacteriology

The infectious organisms of community-acquired pneumonia (CAP) vary according to patient population, host immunity and geographic region, with the most common pathogens including *S. pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus* (25). However, despite the relationship with pneumonia, studies suggest the bacteriology of pleural infections differ from that of pneumonia and have been altered significantly with the institution of antibiotic treatment. In a study of 434 patients with pleural infections, approximately 50% of parapneumonic infections were due to *Streptococcal* species, with the most common being *S. intermedius* [*S. anginosus* (*milleri*) group], followed by *S. pneumoniae*. *Staphylococcus* species were also common and accounted for about 14% of the parapneumonic infections. In this series, another, 20% of parapneumonic effusions were due to anaerobic bacteria (24). Most other series report similar rates of anaerobes (12-24%). However when DNA amplification and research laboratories are used to identify organisms, anaerobes may be present in up to 76% of the cases (14,33-35).

The difference in the bacteriology between pneumonia and pleural infections may be related to the acidic and hypoxic environment of the infected pleural space and bacterial virulence factors favoring certain pathogens (18,24). Furthermore, the difference in bacterial species between pleural infections and pneumonia, along with lack of chest imaging evidence of pneumonia in some patients, have led some experts to question the conventional belief that empyema and pneumonia are inherently related. Hematogenous spread of bacteria from systemic infection or an abdominal process with rapid growth of bacterial in the pleural space, as observed in animal models, offers a

plausible explanation (24,31).

Predictive factors

Aside from inflammation in the lungs and pleural space from direct invasion of bacteria and bacteriologic virulence features contributing to parapneumonic effusion, patient factors and comorbidities also contribute to the pathophysiology of parapneumonic effusion development. A recent study (11), analyzed 4,715 patients with CAP and 882 (19%) had pleural effusions, of which 261 (30%) had empyema or complicated parapneumonic effusion. In a multivariable analysis, no single baseline patient characteristic distinguished patients without pleural effusion from those with uncomplicated parapneumonic effusion. However, five independent baseline characteristics could predict the development of empyema or complicated parapneumonic effusion in patients with pneumonia: age <60 years old, alcoholism, pleuritic pain, tachycardia and leukocytosis. These investigators, and others have found a reduced prevalence of clinical manifestations in older patients, suggesting possible age-related change in the immune response (11,13,36,37). In this cohort, researchers also found patients with a history of tobacco abuse had increased risk of developing a complicated parapneumonic effusion or empyema, whereas, chronic obstructive pulmonary disease and heart failure decreased risk. Diabetes, chronic renal disease and liver disease were not associated with risk of pleural infection in the cohort (11). Similar results have been found in other prospective observational studies of patients diagnosed with CAP (12,13).

Pneumonia is a leading cause of death and pleural infections complicating pneumonia has been established to have considerable morbidity and mortality, with mortality approximately 20% for patients with empyema (16,38,39). This may be related to something inherent about the parapneumonic effusion and/or a more robust inflammatory response. Underlying comorbidities or patient factors may not only contribute to the development of a parapneumonic effusion, but might be the cause of increased mortality.

In a prospective cohort study of 1,906 patients with CAP, Hasley *et al*. (36) found an overall 30-day mortality of 4.9%. Patients with associated pleural effusion had 30-day mortality of 14.7% and those with bilateral effusions had an even higher mortality of 26.0%. In multivariate analysis of radiographic features and clinical characteristics, the presences of bilateral pleural effusions were independently associated with mortality. Other radiographic characteristics including infiltrates involving two or more lobes, the presence of bronchopneumonia, bilateral infiltrates, air bronchograms, postobstructive pneumonia, or an aspiration pattern, had univariate associations with mortality. However, none of these factors were independently associated with death after controlling for confounding variables know to be associated with mortality. The most common comorbidities in this pneumonia cohort were coronary artery disease, chronic obstructive pulmonary disease, and CHF. Mortality rates were greatest for patients with both CHF and bilateral pleural effusions (25.6%).

Why bilateral pleural effusions are associated with mortality in pneumonia is not clear nor is the observation that patients with bilateral effusions and CHF are at greatest risk of mortality. Bilateral effusions in pneumonia patients may be a marker of severe pneumonia or may be attributable to underlying comorbidities. The increased mortality in patients with comorbidities such as CHF may reflect overall health status and those who are more likely to die from co-existing heart disease rather than directly from the pneumonia. Further research is needed (5,12,36).

Management

Management of parapneumonic effusions involves a stepwise approach in addition to appropriate and timely antibiotic treatment. The treatment options include: observation, therapeutic thoracentesis, tube thoracostomy, intrapleural instillation of fibrinolytics, thoracoscopy with breakdown of adhesions and/or decortication, and open drainage procedures (5,6,32). The details of these procedures go beyond the scope of this article.

Summary

Pneumonia can be complicated by the development of a parapneumonic effusion, which has increased morbidity and mortality. Complicated parapneumonic effusion and empyema often necessitate prolonged treatment, longer hospital stay and interventions. Parapneumonic effusions arise from inflammation in the lungs and pleural space from a cascade of inflammatory events including, direct invasion of bacteria and bacteriology virulence features. Patient factors and comorbid illnesses such as heart failure also contribute to the pathophysiology of parapneumonic effusion development.

The evolution of parapneumonic effusions can be divided into three progressive stages: (I) exudative; (II) fibrinopurulent; and (III) organizing stages. These stages can help categorize

effusions into groups in order to evaluate risk of an uncomplicated or complicated course requiring intervention. Clinical data should be collected to classify patients and a stepwise approach be taken in the management.

Acknowledgements

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Advanced imaging in COPD: insights into pulmonary pathophysiology

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Abstract: Chronic obstructive pulmonary disease (COPD) involves a complex interaction of structural and functional abnormalities. The two have long been studied in isolation. However, advanced imaging techniques allow us to simultaneously assess pathological processes and their physiological consequences. This review gives a comprehensive account of the various advanced imaging modalities used to study COPD, including computed tomography (CT), magnetic resonance imaging (MRI), and the nuclear medicine techniques positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Some more recent developments in imaging technology, including micro-CT, synchrotron imaging, optical coherence tomography (OCT) and electrical impedance tomography (EIT), are also described. The authors identify the pathophysiological insights gained from these techniques, and speculate on the future role of advanced imaging in both clinical and research settings.

Keywords: Chronic obstructive pulmonary disease (COPD); respiratory physiology; medical imaging; pulmonary ventilation; respiratory function tests

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality world-wide. Its impact is significant and increasing: COPD is predicted to be the 4th leading cause of death and the 7th leading contributor to the global burden of disease by 2030 (1). A better understanding of its pathophysiology, early detection and effective treatments is therefore imperative.

COPD involves at least two well-defined pathological features, namely parenchymal lung destruction (emphysema) and the loss or narrowing of airways (termed airways disease). The measureable physiological correlate of these changes is airflow obstruction, as indicated by a reduced spirometric ratio [forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC)]. Until recently, these structural and physiological components have been studied in isolation, with inferences made about their relationship.

Advanced imaging techniques allow detailed anatomical and structural data to be acquired *in vivo*. Functional data can also be acquired, often in real-time, and co-registered with the anatomical images. In combination, these imaging data expose a remarkable degree of regional variation in lung function. Depending on the resolution of the images, information can be obtained down to the alveolar level (2). The different modalities vary greatly in terms of temporal and spatial resolution, and each has its own advantages and disadvantages.

In this review, we will describe advanced imaging modalities that are currently in use, either clinically or in a research setting, at varying stages of development. A large focus will be on their role in furthering our understanding of pathophysiology, clinical phenotyping and response to treatment. We will also speculate on the future place of

these techniques among the assortment of tools available to clinicians for managing COPD.

High-resolution computed tomography (HRCT)

X-ray computed tomography (CT) has been in commercial use since 1972 (3), and has been revolutionary in providing insight into pulmonary structure and function *in vivo*. The technology involves an X-ray beam source with a row of detectors positioned opposite—the source and detectors are assembled in a circular arrangement that rotates around the patient through 180 degrees. Measuring the attenuation of narrow X-ray beams as they pass through tissues of varying densities allows the construction of a 2-dimensional (x,y) axial 'slice' through the body. The digitised image slices are comprised of pixels with relative 'densities' [measured in Hounsfield Units (HU)] that are representative of the tissue density in that location. Early scanners used relatively thick, contiguous slices that were obtained along the cranio-caudal (z) axis as the patient was moved stepwise through the scanner.

High-resolution CT achieves its increased spatial resolution by the use of thinner detectors, which allows the effective thickness of the axial slices to be reduced, usually to around 1 mm. The physical size of the X-ray detectors is therefore one of the determinants of resolution, while scanning technique is the other and more dominant determinant of spatial resolution. The high-resolution technique traditionally involves axial or 2-dimensional scanning, i.e., subjects are stationary during a single tube rotation to acquire a single cross-sectional slice. Because of the increased time and radiation required to perform contiguous thin slices, the slices are typically separated along the z axis by an interval of around 10 mm, which minimises total radiation exposure but images only 10 percent of the lung. It is therefore suitable for imaging the lung parenchyma but not for detecting, for example, mass lesions.

These days, almost all CT scans are performed in 'helical' or 'spiral' mode, rather than the older axial technique. That is, the patient is moved continuously through the scanner during tube rotation, effectively producing a 'cork-screw' motion. In this way, 3-dimensional or volumetric data is obtained; the faster the patient is moved through the rotating tube (pitch), the faster the acquisition time and the lower the radiation exposure. However, this effectively produces greater blurring of the images and reduces spatial resolution. Hence imaging technique, including tube

current and voltage, once again has a large effect on spatial resolution.

Post-processing of the raw image data produces reconstructions in three orthogonal planes, which are most commonly displayed as axial images. Reconstruction also involves algorithms to produce images that are optimised for diagnostic viewing, such as 'high-resolution reconstruction algorithms'. These produce sharper but noisier images. Advances in CT technology have led to faster tube rotation, greater detector sensitivity and more rows of detectors – now up to 128 rows. This has greatly reduced acquisition time and reduced breath artifact; computer algorithms are able to correct for artifacts related to image inconsistency and motion (4). Axial slices of any thickness can be reconstructed, down to around 0.5 mm thickness (see *Figure 1*). However, at this level, spatial resolution is determined more by scanning technique (e.g., table speed) than by reconstructed slice thickness. There may therefore be no major advantage in such thin reconstructions. Modern post-processing techniques also allow true 3-dimensional reconstruction of the entire lung, airways and vasculature. Whether these are clinically useful is arguable.

Assessment of emphysema

HRCT is ideal for the detection and characterisation of emphysema (5). Moreover, it is very straightforward to use HRCT images to quantify the extent of emphysema. Older, standardised visual scoring systems to quantify emphysema (6) were subject to a high degree of inter- and intra-rater variability (7). More recent computer-automated quantification tools have removed the subjectivity of scoring (7). Emphysematous lung is represented by image voxels (the unit of a 3-dimensional image dataset) of density less than around –900 HU, which equates to a density of around 0.1 g/mL (water has a density of 1 g/mL). Identification of all voxels of density less than this threshold is a process commonly known as 'density masking', and the volume of the emphysematous lung can be calculated by multiplying the voxel numbers by the known volume of the voxels (8). A commonly used index is the percentage of low-attenuation areas (LAA%), which expresses the emphysema volume as a proportion of total lung volume measured by CT. The LAA% has been shown to correlate with FEV_1 (9-11), diffusing capacity for carbon monoxide (DLCO) (9), the frequency of COPD exacerbations (12), BODE index and quality of life scores (13). These relationships

Figure 1 High-resolution CT scan of a 69-year-old man with allergic bronchopulmonary aspergillosis. Three axial reconstructions of (A) 6 mm, (B) 2.0 mm and (C) 0.625 mm thickness. Note the increasing spatial resolution with decreasing slice thickness. (Helical acquisition with the following settings to maximize spatial resolution: 120 kVp, 548 mAs, tube rotation time 0.5 s, collimator width 0.625 mm, pitch 1.375, voxel dimensions 0.76 mm × 0.76 mm × 0.625 mm).

confirm that the severity of emphysema is a determinant of the severity of airflow limitation as well as the clinical expression of disease.

A proposed clinical application of quantitative CT is in the longitudinal monitoring of emphysema progression. In the ECLIPSE study, which was a 3-year prospective, multi-centre observational study of COPD patients, emphysema progressed and became more extensive over the study period (14). The observations also confirmed that the extent of emphysema predicted the rate of decline of FEV_1 (15). Although it may be useful in identifying the so-called 'emphysematous phenotype', the ECLIPSE study also re-emphasised the marked clinical heterogeneity among COPD patients (16). More work is therefore needed to determine precisely how changes in CT emphysema over time translate into clinically important outcomes.

The LAA% gives an estimate of emphysema quantity, but not its distribution. The importance of emphysema distribution was demonstrated in the NETT (National Emphysema Treatment Trial) study of patients undergoing lung volume reduction surgery (LVRS). In this study, patients who had predominantly upper lobe emphysema (i.e., localised or heterogeneous emphysema, so-named because of the obvious differences in emphysematous and relatively preserved regions in the same lung) had improved survival following LVRS compared to the control group. In contrast, those in whom emphysema was not localised but rather spread out over a large proportion of the lung had poorer clinical outcomes (17).

In addition to this spatial heterogeneity, emphysematous lesions also exhibit so-called 'fractal geometry'. This is measured by identifying emphysematous clusters, i.e., a discreet and isolated zone of emphysema. In COPD, there is a large number of small emphysematous lesions but only a small number of large lesions or cysts. Plotting a cumulative frequency of emphysematous lesion size in log-log space results in a linear relationship with a negative slope (18). The slope of that relationship is the 'fractal dimension', with a more negative slope indicating a more heterogeneous distribution of emphysema zone sizes. As an example of its clinical significance, Coxson *et al.* (19) showed that the fractal dimension derived from the pre-operative CT predicted the change in exercise capacity following LVRS. Although this complex CT assessment of emphysema appears to have some clinical significance, its potential role in routine clinical practice remains unclear.

Assessment of airways

Airways are also visible in HRCT image data, down to approximately generation 6 or 7. In COPD, changes in small airways (terminal and respiratory bronchioles) are considered to be the among the earliest signs of disease (20) and precede the development of emphysema (21). However, CT airway measurement is much more difficult than measurement of emphysema for a number of reasons. Firstly, airway branching is asymmetrical (in terms of length and calibre of bronchi) and hence 'functional' classification of individual airways cannot be made by simple counting of generations (22). Indeed, the small airways (0-2 mm in diameter) can be found anywhere between the 4th and 14th generations (22). Secondly, the measurable parameters of airway geometry (such as airway wall thickness and luminal area) vary greatly by anatomical location (23). Finally, since

the major site of airflow obstruction in COPD is in airways with dimensions less than 2 mm (20,21), the primary area of pathology is generally below the resolution of conventional HRCT.

Early attempts at quantitative analysis of visualised (generally large to medium sized) airways involved manual tracing of internal luminal area (Ai), outer area (Ao) and the calculation of wall area (Aw). Computer algorithms using a density mask have been used to automate this process, whereby circles surrounding the airway lumen are progressively 'eroded' based on density measures until the airway wall and lumen are identified (24). Another method known as the full-width-half-maximum method has been used to identify internal and external wall edges. This principle uses density analysis along radial 'spokes' from the centroid of the lumen, and identifies the wall edge as the point where density is half way between the local minimum and local maximum along each spoke (10). A well-documented problem is that CT measurement systematically over-estimates Aw and underestimates Ai, a feature that alters with airway angle and becomes more pronounced with decreasing airway calibre (24); mathematical corrections can be applied to overcome this error. Automated airway measurements have been validated for larger airways (down to the 6th generation) (25,26), but concerns regarding accuracy continue to limit their use.

Measurement of the small airways is even more problematic. Although large airway wall thickness correlates with symptoms (27) and lung function (10,25) in COPD, and may be predictive of small airway abnormalities (28), direct measurement of small airway geometry by CT has remained elusive. 'Air trapping' is an indirect HRCT measurement of small airway dysfunction, where lung lobules remain inflated due to airway obstruction and hence show a less-than-normal increase in attenuation during expiration, creating a mosaic pattern of attenuation. However, this phenomenon is observed to a degree even in healthy, non-smoking individuals without airflow obstruction (29,30). In COPD, this method is further complicated by the presence of emphysema, which itself shows low-attenuation during expiration (31). Although attempts at quantifying air trapping in the presence of COPD have been made (31,32), more validation studies are needed to determine the best method. For now, at least in the clinical setting, it seems that air trapping on CT will remain a more qualitative marker of small airways disease, and probably adds little to the diagnosis or monitoring in COPD.

Ventilation CT

Although anatomical information from CT is of value for diagnosis and assessment of disease severity, there is likely added benefit when it is combined with imaging-derived measurement of lung function. For example, the distribution of inhaled xenon (Xe) gas (which is radiopaque, and distributes into the airways and alveoli on inhalation) can be measured by its CT attenuation. The CT density of these Xe-containing airspaces increases linearly with Xe concentration (33). In this way, specific ventilation (i.e., ventilation per unit lung volume) can be measured and the regional distribution of ventilation explored. New dual-energy (i.e., two X-ray sources) CT allows both dynamic and static evaluation of regional ventilation, and simultaneous acquisition of anatomical and ventilation images (34). This eliminates the problems of serial scanning, such as differences in breath-hold volume affecting density, and spatial misregistration of the two scans (35). Dual-energy Xe gas ventilation scanning has been shown to reliably quantify both emphysema and airways disease (36).

Limitations of CT

In addition to problems with spatial resolution, CT has other important limitations. For example, there are minimal data regarding normal ranges for airway dimensions (37) and currently no consensus standards for validation and quality control of CT airway measurement—a prerequisite for high-quality, longitudinal studies. Perhaps the most important limitation of CT is the risk posed by ionising radiation, particularly with serial scanning (38). Quantitative measurements using current-generation detectors with low-dose protocols may be acceptable for certain applications (39) but the reduced signal-to-noise ratio poses an additional challenge when assessing small airways (40,41).

Ultra-high-resolution imaging

Like all diagnostic tools, imaging techniques need to be verified against a gold standard test. In the case of COPD, this would be histopathological evaluation of lung tissue. However, even as a gold standard, histopathology itself has problems: tissue changes from fixation, cutting and drying as the specimen is processed cause measurement error; the small size of specimens may introduce sampling error; and analysis is generally performed in two (or even single) dimensions, giving only estimates of the 3-dimensional
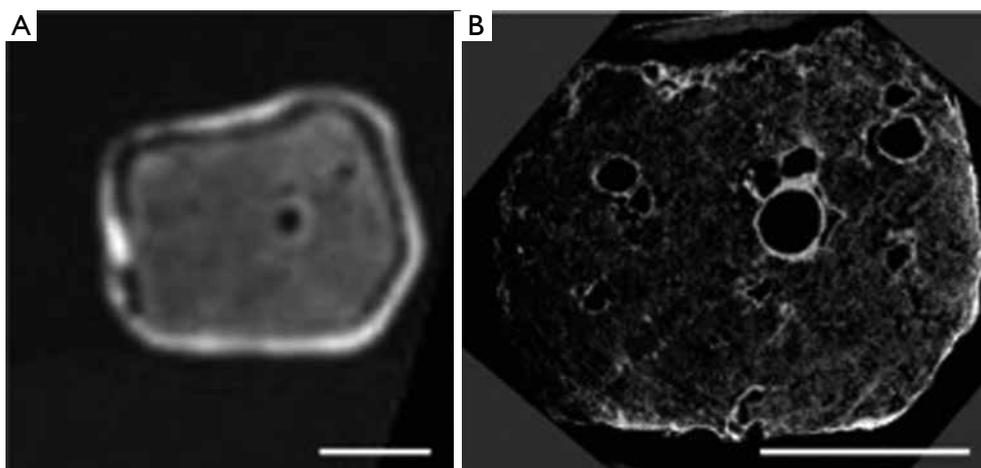
Figure 2 Micro-computed tomography (micro-CT) of a 2 cm pig lung cube. Lungs were inflation-fixed in formalin steam prior to scanning. (A) Cross-sectional high-resolution CT image and (B) corresponding micro-CT image of the same region of interest. Exquisite detail is seen with micro-CT. Scale bars =1 cm.

structure (42). The use of ultra-high-resolution imaging techniques is aimed at countering these problems.

Micro-computed tomography (micro-CT)

Micro-CT is similar to conventional CT in that it uses an X-ray source and detectors that are arranged around the study object. Like conventional CT, full volumetric data is captured. However, the source and detectors are brought much closer to the specimen, and the specimen itself is rotated while the X-ray source and detectors stay stationary. This allows exceptionally higher resolution (down to 1 µm per voxel), which is ideal for studying the lung microstructure including that of the small airways. The trade-offs are that only small specimens, i.e., excised tissue or small animals, can be imaged. Furthermore, the samples are exposed to high radiation doses that are damaging to living tissue.

Micro-CT was first used to image lung parenchyma by Watz *et al*. (43), who used a hot formalin vapour fixation technique and silver nitrate staining to provide the necessary contrast. This provided spectacular images of alveoli and terminal airways, and even allowed a "virtual bronchoscopy" through an alveolar duct (43). The technique has subsequently been validated against light microscopy in mouse lungs (44). It has also been used to generate gold-standard airway measurements from explanted lung to calibrate 3-dimensional airway measurements made by whole body HRCT (45). *Figure 2* demonstrates the

spectacular detail obtained using micro-CT of the lung. In COPD, micro-CT has been used to demonstrate the loss of terminal bronchioles in early-stage disease, which has been suggested to precede the microscopic emphysematous destruction of the alveoli (21).

Although living, *in vivo* human studies are precluded (due to both specimen size and the radiation dose), micro-CT has provided fascinating insights into the structural changes in COPD. The technology, in its currently form, will likely remain limited to research. It may play a future role in, for example, developing disease-modifying therapies in animal models.

Synchrotron imaging

This form of imaging utilises the properties of particle beams, e.g., electrons, in a particle accelerator. The particles are in continuous motion at near the speed of light, held in line by electromagnetic fields. When the particles are accelerated further they emit X-rays with a wide energy range, allowing a wide range of samples to be imaged, and a high photon flux, which allows fast acquisition times. The result is an image with resolution in the 1-10 µm range (46), which is ideally suited to studying the microstructure of fine tissues such as the lung.

The technique has been used to define the structure of both mouse (47) and human (48) lung acini *ex vivo*, as well as *in vivo* whole mouse lungs (49). The high temporal resolution also makes functional imaging possible,

including pulmonary acinar mechanics (50) and regional ventilation (51,52). In COPD mouse models, it has been used to identify early emphysematous changes (53,54). While the anatomical detail is impressive, synchrotron imaging is yet to provide significant functional information on human lungs, being hampered by the limitations of specimen size (necessitating excised tissue or small animals only), radiation damage to tissues, and the need for a particle accelerator.

Nuclear medicine imaging

Unlike CT imaging methods, which are based on the relative absorbance of radiation transmitted through the tissues from an external source, nuclear medicine techniques utilise tracers that emit radiation and are introduced into the organs. This has been used to image a variety of body tissues and organs, including bone, the heart and the brain. Nuclear medicine scanners are in routine clinical use for the diagnosis and staging of malignancy. They have also long been used for the diagnosis of pulmonary embolism (PE)—this involves intravenous injection of radioisotopes to the pulmonary vasculature and inhalation to the peripheral airspaces, thus giving functional images of ventilation and perfusion.

Positron emission tomography (PET)

PET is a 3-dimensional nuclear imaging technique, which utilises radioisotopes that emit positrons as they decay. A positron is a sub-atomic particle found in the nucleus, with the same molecular weight as an electron. As the positron makes its way out of the nucleus, it encounters a free electron—these two oppositely-charged particles combine and 'annihilate' each other. In the process, two identical beams of gamma-radiation are emitted at 180 degrees to each other—beams detected at or very near the same time are considered to be 'coincident' i.e., from the same source. The location of the source particle can therefore be determined geometrically from coincident beams, being located on a straight line between the two detectors. However, this localisation is affected by beam scatter, the presence of random coincidences, and by attenuation as the beams travel through tissues of different densities. Corrections for this image noise can be made during image processing, which includes the use of a tissue density map i.e., a CT scan. Many scanners incorporate multi-detector CT (PET-CT) so that, in addition to providing a tissue density map for attenuation correction, organ function

can be superimposed onto the CT images. This image co-registration has an obvious application in oncology for localisation of active tumour cells for targeted treatment.

The most commonly used PET radioisotope is fluorine-18, which has a half-life of approximately 110 minutes. This isotope is attached to fluorodeoxyglucose (FDG, a glucose analogue) to form the radiotracer 18F-FDG. The radiotracer is taken up by metabolically active tissue—a property used to identify cancerous tissue in the lungs and surrounding structures. In COPD, 18F-FDG has been used to demonstrate an increase in neutrophilic inflammation in the lungs compared to controls and to those with alpha-1 antitrypsin deficiency-associated emphysema (55). This provides interesting insights into COPD pathophysiology and is a non-invasive, *in vivo* measurement. However, it is also potentially useful in studies of new therapies targeting neutrophils, given their role in the pathogenesis and pathophysiology of COPD (56).

Regional ventilation can also be measured by PET using the PET isotope nitrogen-13 (13NN) gas dissolved in saline. A peripheral venous injection of this tracer enters the lung via the pulmonary arterial circulation. Due to its low tissue solubility, the 13NN then rapidly diffuses across the alveolar membrane. An initial breath-hold during injection allows the 13NN to enter the lung in direct proportion to blood flow, which allows measurement of regional perfusion. Regional ventilation can subsequently be measured by the decrease in 13NN activity over time as the subject breathes, clearing the 13NN in direct proportion to ventilation (57). Combining this information thus produces the regional distribution of lung ventilation/perfusion ratios (V/Q). Quantification of regional V/Q by PET has been shown to correlate closely with global measures of gas exchange such as arterial partial pressure of oxygen (PaO$_2$) (58). Using this 13NN technique in COPD, Brudin and colleagues (59) reported that high V/Q tended to be more common in subjects with an emphysematous phenotype, whereas low V/Q was more common in those with a small airways disease phenotype. This is consistent with the archetypal concept of 'pink puffers' and 'blue bloaters'.

While much of the focus in COPD is on airways disease and changes in regional ventilation, there has been an increasing focus on the role of the pulmonary vasculature in this disease. Vidal Melo *et al*. (60) found that regional heterogeneity in Q was increased in patients with mild COPD compared to healthy controls, in a manner that was independent of changes in regional tissue density. This very interesting finding suggests that regional changes in

pulmonary blood flow, perhaps due to inflammation, may precede lung parenchymal changes in COPD. This may have utility as a biomarker for early disease.

One limitation of PET, with its short half-life radioisotopes, is the need for a cyclotron and radiopharmaceutical formulation often on-site. Additionally, for repeated studies, the radioisotope has to decay enough to avoid signal contamination. Adjustments following a 'baseline' scan prior to repeat administration help overcome this problem, however rapid, repeat testing is generally not possible. The spatial resolution of PET does not allow imaging of individual gas exchange units, although it is probably sufficient to separate physiologically meaningful differences in regional ventilation.

In spite of these limitations, and its relatively recent inception, PET may have a significant future in the study of COPD. The recent findings regarding regional distribution of blood flow may provide insight into the role of vascular remodeling, especially with regard to longitudinal changes and therapies targeted at this process.

Single-photon emission computed tomography (SPECT)

SPECT is similar to PET in that a radiotracer is introduced to the body, and the radiation it emits is detected externally. However there are several key differences. SPECT radioisotopes emit a single gamma-beam as they decay, as opposed to the two gamma beams emitted simultaneously from PET isotopes. This results in a lower radiation exposure to the patient, at the expense of an increased acquisition time per image. Increased scanning time may decrease resolution due to movement artifact, although there have been attempts to overcome this with breath hold/respiratory gating (61). The spatial resolution of SPECT is less than that of PET, however it is more widely available, and SPECT radiotracers are easier and cheaper to manufacture. SPECT has been a major advance in nuclear imaging for suspected PE (62), as opposed to the traditional planar lung scintigraphy. The more recent appearance of SPECT-CT fusion has helped overcome a lot of the resolution and anatomical registration problems, and can provide true 3D assessment of regional lung function (63,64).

In addition to its diagnostic role for PE, SPECT can give us insights into pulmonary physiology, both with respect to ventilation and perfusion. Perfusion scanning is generally performed using 99m-technecium labeled macro-aggregated albumin (^{99m}Tc-MAA), which lodges in the pulmonary circulation after peripheral injection. Ventilation

scanning requires inhalation of gaseous radioisotopes or radiolabeled particulate aerosols. A true gas distributes throughout the whole lung, and differences in its regional distribution reflect differences in regional ventilation. Dynamic SPECT could therefore potentially give information on the time course of ventilation in different lung regions. Both ^{81m}Kr (65) and ^{133}Xe (66) have been used to demonstrate ventilation heterogeneity in COPD.

Unlike true gases, particulate aerosol tracers are 'deposited' in the lung and have the advantage that imaging can be performed without the tracer continuously redistributing. Also, aerosols will not distribute by collateral ventilation between lung units, which is increased in COPD compared with healthy lungs. However, aerosol particles of a diameter 0.5-1 µm are 1,000 times larger than gas molecules and are therefore transported by convective ventilation only (67). The distribution of radioaerosols therefore neglects diffusive ventilation, which is the predominant mode of gas transport within acini beyond the terminal bronchiole (68). In COPD, airway narrowing and emphysematous destruction likely brings the convection-diffusion front more centrally so that a larger volume of the lung ventilates by diffusion compared with healthy lungs. Therefore, the interpretation of inhaled radioaerosols distributions in COPD should take these physiological changes into account (69).

A commonly used aerosol tracer is 99m-technecium-labelled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA). The generated particle size is around 1 µm but this increases on entry into the airways due to agglomeration. These larger particles deposit onto large airways, particularly at airway branch points, causing 'hot-spots' on the ventilation image (70). Technegas is a ^{99m}Tc-labelled, aerosolised ultra-fine carbon particle of approximately 200 nm diameter. It is used routinely in Australia (where it was invented) and other countries in V/Q scanning for the diagnosis of PE. Due to its small particle size, the distribution of Technegas approximates that of a true gas (71), even in the presence of severe airflow obstruction (72) (*Figure 3*). Technegas deposits more homogenously, and is less susceptible to central airway deposition or movement after inhalation, than ^{99m}Tc-DTPA (73).

Regional V/Q ratios are heterogeneous in COPD due to the variable effects of inflammation and tissue destruction on lung parenchyma, small airways and blood vessels. Jogi *et al*. (74) reported significant relationships between SPECT-derived V/Q ratios and both airflow obstruction measured by spirometry and emphysema severity on CT. In
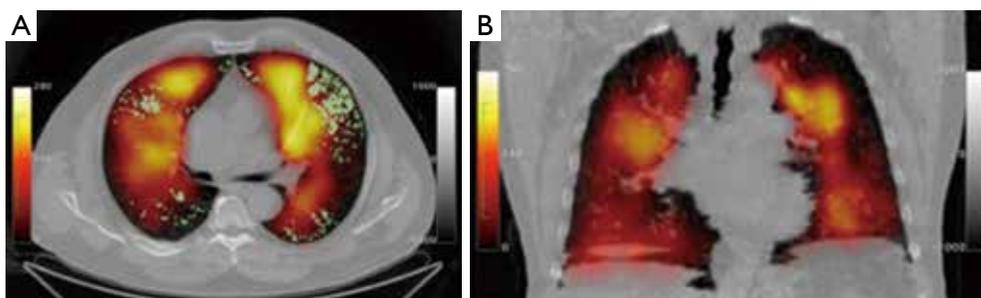
Figure 3 Technegas ventilation SPECT/CT fusion images of a 68-year-old man with moderately severe COPD. (A) Axial dimension, with well-ventilated areas (bright yellow), less ventilated areas (red) and non-ventilated areas (black). Green indicates emphysema determined by a CT density mask, with pixel density less than –910 HU. Note that poorly ventilated areas tend to correspond to areas of emphysema. Some non-ventilated areas are not associated with emphysema, suggesting airway obstruction without macroscopic parenchymal destruction in these areas; (B) coronal reconstruction of ventilation map.

patients with COPD, Suga *et al.* (75) found that automated, quantitative analysis of V/Q distribution by SPECT was more sensitive at detecting early emphysema than the corresponding CT density mask. The standard deviation of the V/Q profile (i.e., dispersion of V/Q ratios) could differentiate the GOLD spirometric classes of severity, and also correlated well with the measured alveolar-arterial oxygen gradient (A-aDO2), a global measure of V/Q inequality (75).

There have been a number of intervention studies in COPD patients using nuclear scintigraphy techniques, including SPECT, to predict and measure clinical success. For example, SPECT imaging has been shown to predict post-operative lung function following surgery for lung cancer. Sudoh *et al.* (76) demonstrated that perfusion SPECT/CT was as accurate as the segment-counting technique (77) but was less affected by the presence of severe emphysema (76). In emphysematous patients undergoing LVRS, Inmai and colleagues (78) found that LVRS improved ventilation distribution as measured by Technegas SPECT, not only in the surgical field but also in the contralateral lung. Recently, Argula *et al.* (79) performed a retrospective analysis of data from the VENT endobronchial valve study (80) to investigate the effects of baseline lobar perfusion on outcomes following endobronchial valve placement. In this analysis, target lobe perfusion was quantified from ^{99m}Tc-MAA perfusion images taken prior to stent insertion. The patients were dichotomised as 'high' or 'low' baseline lobar perfusion. Post-procedure, the low perfusion group were found to have a significantly greater increased in 6-minute walk distance at 6 months, which was independent of the degree

of emphysematous destruction in that lobe. The authors postulate that the redistribution of blood flow seen in the 'high' baseline perfusion group may explain their poorer exercise performance (79). This study used planar scintigraphy, and it is possible that similar studies using the more sophisticated SPECT/CT may shed further light on this interesting finding. To date, there are no published studies using SPECT ventilation imaging to assess the effects of treatments aimed at improving the ventilation patterns in COPD, including bronchodilators, inhaled corticosteroid therapy, bronchial stents or intrapulmonary thermal treatment.

In summary, there have been many technical advances in SPECT imaging of lung ventilation and perfusion. There are potentially many research questions in COPD to which SPECT imaging could be applied, particularly because of its relative wide availability in large centres. Future studies will inform eventual clinical applications in COPD. Although PET ventilation imaging could eventually be preferred over SPECT, the current availability and cost of PET may limit its use.

Magnetic resonance imaging (MRI)

MRI has the advantage of not requiring ionising radiation. Its usefulness for lung imaging has traditionally been limited by technical factors and, consequently, there has been much less MRI lung imaging in research and clinical practice compared with the modalities already discussed. The technique employs large magnetic fields, which alter the behavior of individual atoms. Conventional MRI utilises the nuclear spin properties of hydrogen atoms, found in

abundance in water. In their natural state, hydrogen ions (or protons) spin on an axis in random orientations. The application of a strong and uniform magnetic field causes them to align parallel or anti-parallel to the field. When the magnetic field is switched off, the spinning protons return to their natural state and in the process release a burst of radiofrequency energy. It is this energy that is detected by the MRI scanner, and is used to construct the image. MRI has excellent anatomical resolution in tissues of high water content, such as the brain. However the lung, being mostly air, has a low density of hydrogen ions, meaning low signal intensity and poor signal-to-noise ratio. Conventional proton MRI images of the lung therefore have low contrast and contain little meaningful information. Additionally, being comprised of air-filled alveolar sacs, the lung has millions of air-tissue interfaces. Each of these causes decay of the radiofrequency signal as it travels through the lung, known as 'susceptibility artifact', which contributes to poor signal intensity (81). Such inherent limitations have left the lung relatively unexplored by MRI. However, the large potential for MRI imaging of the lung has been recognised for decades (82) and thus many attempts have been made to overcome these barriers.

Inhaled noble gas MRI

Inhaled noble gases have been used to overcome the aforementioned limitations (83). Unlike particulate aerosols, gases undergo 'self-diffusion' by random Brownian motion, where the gas molecules continually move further apart until stopped by a physical boundary. The speed and direction of movement is determined by the physical properties of the gas, and the likelihood of colliding with a neighbouring gas particle i.e., the local concentration of the gas. An example of a noble gas ventilation agent is hyperpolarised helium-3 which, unlike air, is highly excitable by a magnetic field and thus provides excellent MRI contrast. It diffuses freely in air at a rate of 0.88 cm^2/s, which means that, over a timecourse of 2 ms, an individual molecule will travel 0.59 mm. Given that the typical acinar size is 0.3 mm, the diffusive movement of a molecule of ^{3}He in an acinus will be limited by the alveolar boundaries within the 2 ms timeframe—that is, its diffusion will be 'restricted' from 0.88 to 0.2 cm^2/s. The restricted gas molecule movement is measurable by MRI, and is known as the 'apparent diffusion coefficient (ADC)'. A high ADC indicates that the alveolar walls are further apart, i.e., there is alveolar destruction or acinar expansion, which is an early sign of emphysema (84).

The technique has been validated against histological specimens (2). It is increased in smokers who still have a normal FEV$_1$, suggest that alveolar expansion and early emphysema are present even without clinical manifestation of disease (85). The ADC also correlates very closely with standard lung function measures including FEV$_1$/FVC ratio, TLC and RV (85). In more advanced disease, the mean ADC correlates strongly with FEV$_1$ (86) and DLCO (87), in fact more strongly than HRCT measures of emphysema (86,87).

From a functional perspective, ventilation can also be assessed using the properties of inhaled noble gases. Inhalation of the hyperpolarised gas distributes reasonably homogeneously in healthy young adults, but is heterogeneous in otherwise healthy elderly subjects (81). Regions of absent MRI signal, which indicate non- or poorly-ventilated lung units, have been shown to correlate with emphysema that is detectable by CT or the ADC (88). Ventilation defects may also be observed even in the absence of anatomically gross emphysema visible by CT (89,90). In this case, small airways disease, mucous plugging or a combination of both small airways diseases and microscopic emphysema are possible explanations for absent ventilation. This suggests that, like PET and SPECT, functional disturbances measured by MRI may be more sensitive markers of early abnormalities in COPD, compared with anatomical imaging by HRCT.

The ability of hyperpolarised ^{129}Xe to diffuse across the alveolar membrane into the circulation allows gas exchange to be measured. There is a large chemical shift of ^{129}Xe between the gas compartment, the dissolved (tissue and plasma) compartment and the red blood cells (91). By detecting the change in resonance frequency between these compartments, measures of the alveolar membrane thickness (91) and blood uptake (92) can be made. This technique has been used to demonstrate the influence of posture on regional perfusion heterogeneity (93) as well as ventilation heterogeneity (94) in COPD.

Oxygen-enhanced MRI

Although the use of hyperpolarised noble gases has greatly advanced the use of MRI for lung imaging, these gases are expensive to use, requiring specialised laser polarising equipment and dedicated detectors. ^{3}He in particular is in limited and restrictive supply. There is therefore a need for simpler and less expensive contrast agents. Oxygen was suggested as a MRI contrast agent by Edelman (95) over 15 years ago as a way of overcoming the inherent

limitations of conventional proton MRI and avoiding the problems of hyperpolarised gases. Ohno and Hatabu (96) have written a detailed review of the theory and application of oxygen-enhanced MRI. In basic terms, molecular oxygen is weakly paramagnetic and, in the concentrations found in air and in blood, provides little MR signal. The inhalation of 100% oxygen produces a high concentration of oxygen in alveolar tissue and in blood, where is it predominantly dissolved in plasma. The result is an increase in the signal intensity, which then allows visualisation of the pulmonary parenchyma. The difference between room-air and oxygen-enhanced images represents ventilation to that area. Edelman's original publication (95) clearly showed ventilation defects in a patient with emphysema. Ohno and colleagues (97) showed that oxygen-enhanced MRI was as good as CT at quantifying pulmonary emphysema across a wide range of severities, and correlated reasonably well with FEV_1 and DLCO. Recent work in subjects with COPD has shown increased heterogeneity of V/Q distribution measured by oxygen-enhanced MRI even in those without CT-defined emphysema (98), which varies with the severity of COPD (99).

The advantages of oxygen-enhanced MRI (OE-MRI) are that it is a simple, low-cost and safe alternative to hyperpolarised gas MRI. One of the limitations is that the gas itself is not directly visualised, but rather the tissue and blood, so that OE-MRI is only an indirect measure of ventilation. Another limitation is that the absorption of oxygen by circulating blood removes it from the lung unit, meaning there would inevitably be a difference between the wash-in and wash-out phases if they were measured. Additionally, the administration of 100% oxygen to patients, particularly those with advanced COPD, may alter the fundamental pulmonary physiology that we are attempting to measure (96).

In summary, MRI is increasing our current understanding of regional ventilation in COPD, whilst overcoming the limitations of ionising radiation associated with other functional imaging modalities. Cost and availability of the gases, polarisers and research scanning time will likely remain major constraints. Therefore, OE-MRI may be more practical in terms of clinical application. More studies are needed to build on the limited treatment (100,101) and longitudinal (102) data available to date.

Emerging imaging modalities

Optical coherence tomography (OCT)

OCT has emerged from the field of interventional pulmonology. It involves the measurement of lung structure from an endobronchial approach. Analogous to B-mode ultrasound but utilising light waves rather than sound waves, OCT involves the insertion of a near-infrared optical probe into the airway, with a sensor to detect back-scattered and reflected light waves. A detailed description of the physics of OCT is present in Huang's seminal review of the topic (103). OCT images have sufficient resolution to distinguish between different tissue types within the airways, i.e., mucosa, sub-mucosa, lamina propria, cartilage, airway smooth muscle and alveoli. This ability of OCT can therefore potentially identify malignant tissue at the time of bronchoscopy, where the structural components of tissues are altered in their organisation, content and reflective properties (104).

Although there are few studies in subjects with COPD, OCT is ideally placed to measure the anatomical properties of small airways, being limited only by the physical reach of the probe and by the need for repeated measurements in different areas to obtain representative sampling. Miniaturised probes can be introduced down to the level of the terminal bronchiole (105,106). Coxson and colleagues demonstrated an excellent correlation between airway dimension measured by OCT and by CT (107). OCT may give more accurate measurement of airways size since, in this study, CT-measured dimensions tended to be larger than the OCT measurements. Furthermore, OCT airway dimensions measured at the 5th generation bronchi showed a strong negative correlation with the subject's FEV_1, and had greater discriminatory power for airflow obstruction than CT measurements (107). There was also an increase in %wall area and an increase the density of subepithelial structures in subjects with a lower FEV_1 (107). Kirby *et al.* (108) reported a strong negative correlation between airway wall area and FEV_1 in males COPD subjects but not in females, which is an interesting observation that may be relevant to the observed differences in disease behavior between the sexes (109,110).

OCT shows promise as a very useful tool for relating structural and functional changes in COPD *in vivo*. There are very important advantages of high resolution, the ability to measure small airways and the lack of ionising radiation, but it is nevertheless an invasive procedure requiring at least conscious sedation. The potentially important and novel information on small airways means that it will likely be increasingly used in research and clinical practice as the technology improves and becomes more accessible.

Electrical impedance tomography (EIT)

As its name suggests, EIT measures differences in impedance to the flow of an electrical current through different tissues. A typical setup involves a set of surface electrodes, usually 16 to 32, positioned around a body structure. Through a pair of electrodes (the 'drive pair') a small current is applied. The potential difference between each pair of adjacent electrodes is then recorded, and hence the resistivity or impedance at that location can be determined. The process is repeated with each pair of electrodes acting as the drive pair, and a spatial map of resistivity is developed. This technique is ideally suited to pulmonary monitoring for several reasons. Firstly, the lungs, being filled with air, have naturally high impedance and are subject to large changes in volume during respiration. This gives a relatively large 'swing' in impedance that can be used to monitor breathing patterns and interventions. The change in electrical lung impedance is proportional to the change in gas content, which has been validated against other imaging modalities (111-113). Secondly, a decrease in impedance from initially high values could be used to detect focal consolidation/collapse or more diffuse changes in, for example, acute respiratory distress syndrome (ARDS). Thirdly, the short acquisition time provides enough temporal resolution for real-time monitoring of the lungs over long periods, as opposed to quasi-dynamic imaging of ventilation CT or nuclear medicine. Finally, being small and portable, EIT can be used in a variety of physical settings.

Even though the potential clinical utility of EIT respiratory monitoring has been recognised for many years (114), the pulmonary application of EIT has so far been largely limited to the intensive care setting. For example, EIT has been used to develop protective ventilation strategies by optimising positive end-expiratory pressure (PEEP) to minimise regional hyperinflation and collapse (115). In a case report of a patient with COPD undergoing mechanical ventilation, Mauri *et al.* (116) could optimise ventilator settings to overcome intrinsic PEEP and decrease gas trapping measured with OCT. More recently, the technique has been used to explore other obstructive airways diseases. Zhao and colleagues (117) showed that, in patients with cystic fibrosis, regional airway obstruction measured by OCT correlated with a CT composite index of bronchiectasis severity, mucous plugging, parenchymal opacity and hyperinflation in the same lung region.

The most detailed physiological study using EIT in subjects with COPD was recently published by Vogt *et al.* (118). EIT was used to measure the regional distribution of tidal volume, inspiratory vital capacity and FEV_1 during a forced expiratory manoeuvre. Ventilation heterogeneity between regions was quantified as the coefficient of variation. COPD subjects showed greater ventilation heterogeneity than either young or older healthy subjects. Importantly, the measurements were able to discriminate between healthy and COPD subjects even during quiet tidal breathing.

EIT therefore represents an exciting new technique for assessing regional ventilation in COPD. It is a simple, portable, radiation-free, real-time measurement that would be well suited to dynamic physiological studies. More work is needed to determine its role in, for example, the early detection of disease and for treatment/intervention studies.

Conclusions

Our understanding of the pathophysiological mechanisms in COPD is increasing with the new era of imaging tools that are available. There is a greater recognition of the complexity of lung mechanics and regional ventilatory abnormalities in this clinically heterogeneous disease, and advanced imaging techniques are at the forefront of this investigation. As older techniques are refined, and new techniques are developed, the information we gain from advanced imaging in COPD is likely to expand exponentially. The major limitation of ionising radiation exposure is being overcome by advances in technology, which minimise radiation dose while increasing image quality. These modalities are likely to become increasingly important in drug design and delivery, and offer the chance to monitor the impact of such therapies over time. Ultimately, the aims of COPD research should be directed towards modifying the natural history of the disease. We believe the role of advanced imaging techniques in detecting disease in its earliest stage is paramount, as this is the stage at which potentially disease-modifying interventions are likely to have the greatest impact.

Acknowledgements

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Advanced therapies for COPD—What's on the horizon? Progress in lung volume reduction and lung transplantation

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Abstract: Advanced chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity. Treatment options beyond conventional medical therapies are limited to a minority of patients. Lung volume reduction surgery (LVRS) although effective in selected subgroups of patients is not commonly undertaken. Morbidity associated with the procedure has contributed to this low utilisation. In response to this, less invasive bronchoscopic lung volume techniques are being developed to attempt to mitigate some of the risks and costs associated with surgery. Of these, endobronchial valve therapy is the most comprehensively studied although the presence of collateral ventilation in a significant proportion of patients has compromised its widespread utility. Bronchial thermal vapour ablation and lung volume reduction (LVR) coils are not dependent on collateral ventilation. These techniques have shown promise in early clinical trials; ongoing work will establish whether they have a role in the management of advanced COPD. Lung transplantation, although effective in selected patients for palliation of symptoms and improving survival, is limited by donor organ availability and economic constraint. Reconditioning marginal organs previously declined for transplantation with ex vivo lung perfusion (EVLP) is one potential strategy in improving the utilisation of donor organs. By increasing the donor pool, it is hoped lung transplantation might be more accessible for patients with advanced COPD into the future.

Keywords: Chronic obstructive pulmonary disease (COPD); lung volume reduction surgery (LVRS); bronchoscpic lung volume reduction; lung transplant

Introduction

Chronic obstructive pulmonary disease (COPD) is the 4th leading cause of mortality also conferring significant adverse impact on the quality of life for millions of people world wide (1). Goals of treatment are avoidance of disease progression by cessation of noxious particulate exposure, improving exercise capacity by participation in pulmonary rehabilitation, prescription of pharmacotherapy and reducing exacerbation rate (2). Despite these measures a large proportion of patients continue to experience functional impairment and diminished quality of life with consequential economic and social burden (3). This article will explore advanced therapies and surgical interventions for patients who remain impaired despite optimal medical care. The mainstay of treatment options are:

(I) Lung volume reduction surgery (LVRS);
(II) Lung transplantation.

Although yet to be integrated into widespread clinical practise, bronchoscopic methods of lung volume reduction (LVR) are currently being developed. These potentially represent a less invasive, more accessible treatment option for advanced emphysema.

Lung volume reduction (LVR) practises

Physiological basis for LVR

Airway obstruction and emphysema both cause hyperinflation leading to alterations in both lung and

chest wall mechanics (4). The combination of impaired gas exchange, unfavourable lung mechanics at high volume and respiratory muscle inefficiency (due to the respiratory muscles being placed at a mechanical disadvantage) lead to a substantial (and unsustainable) increased work of breathing. Loss of elastic recoil and dynamic airway closure during expiration cause increases in intrinsic PEEP and gas trapping. In these circumstances greater respiratory effort is required to overcome these loads to achieve similar alveolar ventilation. The resulting hyperinflation further exacerbates the problem by reducing respiratory muscle efficiency through diaphragmatic flattening. These physiological alterations result in symptoms of dyspnoea and reduction in exercise capacity. LVR techniques aim to improve respiratory mechanics by resecting, collapsing or obliterating areas of diseased lung making a poor contribution to gaseous exchange. The remaining lung fills the space restoring elastic recoil, reducing dynamic airway closure and gas trapping. The resulting decrease in residual volume returns the diaphragm to a favourable position for efficient ventilation (5).

Lung volume reduction (LVR) surgery

The National Emphysema Treatment Trial (NETT) continues to be the sentinel research underpinning current LVRS practise, defining patient populations for which the intervention confers benefit (6). Prior to this, case series and small randomised trials had suggested benefit (7,8) although patient numbers were modest. Wider concern was voiced about unacceptable mortality and morbidity associated with the procedure (9). The study was designed in response to these uncertainties (10).

The NETT trial randomly assigned 1,218 patients to either LVRS or best medical treatment using exercise capacity and mortality as primary outcome measures. Inclusion criterion included the presence of severe airway obstruction (FEV_1 <45%), gas trapping (RV >150%) and hyperinflation (TLC >100%). All patients underwent pulmonary rehabilitation prior to trial entry.

The early results from the trial defined a patient population (n=140) at high risk of mortality, reaching 16% at 30 days P<0.001 (11).

- FEV_1 <20% predicted and;
- DLCO <20% or homogeneous emphysema pattern.

The presence of these features continues to be an absolute contraindication to LVRS. Such patients randomised to the control group also had poorer prognosis; these clinical characteristics are therefore used within the current transplant guidelines for selection of appropriate patients.

Even after exclusion of high risk patients, NETT did not demonstrate a survival advantage between patients managed medically and surgically. Mortality results for "non-high risk" patients were dependent on post-hoc subgroup analysis stratified by the pattern of emphysema and patient's exercise capacity. Maximal workload at cycle ergometry was used to define exercise capacity-low exercise capacity being less than 40 Watts for males and 25 Watts for females based on sex specific normal values.

The sub-groups were:

(I)   Upper-lobe predominance, low base-line exercise capacity (n=290);
(II) Upper-lobe predominance, high base-line exercise capacity (n=419);
(III) Non-upper-lobe predominance, low base-line exercise capacity (n=149);
(IV) Non-upper-lobe predominance, high base-line exercise capacity (n=220).

Of the four subgroups, only group 1 characteristics conferred a survival benefit during initial follow-up. Over an initial mean follow-up of 29.2 months, these patients undergoing LVRS had a significantly reduced risk of death (P<0.005). No benefit in survival was observed for those patients with non upper lobe emphysema regardless of their exercise capacity. The second primary endpoint of exercise capacity, did favour patients undergoing the procedure. A total of 52% of surgical patients improved exercise capacity defined as any improvement in cycle ergometry from baseline at 6 months compared to 20% of controls (P<0.001). This benefit extended to 24 months although the effect did diminished over time (31% in the surgical group compared to 10% controls had sustained improvement at 24 months).

Long term follow-up of the patient cohorts (12) confirmed the survival benefit to 5 years in the patients with upper-lobe emphysema and low exercise capacity (relative risk 0.67, P<0.003). Again, no survival advantage was demonstrated in the remainder of patients groups. The additional suggestion from this longer term data is the consideration of patients with upper lobe disease and high baseline exercise capacity as a palliative procedure. Significant improvements in quality of life as assessed by the St George's Respiratory Questionnaire (SGRQ) were seen to 5 years.

The long term benefit in the selected patients above must be tempered with shorter term risk of surgery. The original study reported a 90 day mortality of 5.2% in non-high risk patients compared to 1.5% of those patients undergoing

medical therapy. This higher mortality was not seen in the upper lobe predominant low exercise capacity patients for whom the procedure should be considered (2.9 % 90 day mortality *vs.* 3.3% within the control group). Airleak occurred in 90% of patients (median duration 7 days) with 12% persistence at 30 days. Of patients undergoing LVRS, 28.1% remained hospitalised at 30 days. Airleak was universal in those patients not surviving 30 days although the low mortality rate at this time point (3.6%) meant a statistical association was not observed. Nevertheless, higher rates of adverse outcomes (pneumonia, ICU readmission, longer length of stay) were seen in patients with airleak (13). These peri-operative risks and the associated cost implications have contributed to the quest for less invasive bronchoscopic techniques for achieving LVR.

Surgical technique and considerations

The large numbers of patients enrolled in NETT provided an opportunity to compare techniques and outcomes (13,14). Individual centres had the option of using either video assisted thoracoscopic surgery (VATS), median sternotomy or internally randomising patients to either. Of the 552 patients randomised patients who underwent surgery, 69% underwent median sternotomy, with the remainder mostly undergoing a VATS procedure. Choice of operation did not affect mortality outcomes although VATS was associated with shorter ICU and hospital stay with consequential reduced cost (14).

The technique is usually a non-anatomical wedge resection aiming for LVR of 20-30% rather than an anatomical lobectomy (15). Staple lines are a common source of airleak. Prior small non-randomised and randomised studies had suggested that buttressing-reinforcement of stable lines with bovine pericardium or PTFE reduces length of stay (16) and airleak duration (17) with the practise widely applied amongst NETT patients. Patient factors rather than operative technique seemed to have a larger influence on outcome in the NETT cohort. There was no difference in proportion of patients with airleak or its duration when comparing procedure type or buttress material. Longer duration of airleak was associated with lower DLCO and FEV_1, Caucasian ethnicity, use of inhaled steroids, pleural adhesions and upper lobe disease (13).

Non surgical methods for LVR

A number of bronchoscopic interventions have been proposed for non-surgical LVR (18-22). Facilitating LVR bronchoscopically may negate some of the risk associated with surgery, reduce inpatient stay for the procedure and potentially reduce the associated costs. Trial data comparable to the NETT study is not currently available for the majority of these interventions.

For the majority of these techniques, the NETT results have been extrapolated so that patients most likely to benefit can be targeted. Patients identified as 'high risk' by NETT criterion are usually excluded. Likewise most of the existing studies focus on heterogeneous emphysema distribution, usually in the upper lobes. Homogenous emphysema has been addressed with interventions such as airway bypass-endobronchial fenestrations with stenting and LVR coils (LVRCs). The aim of airway bypass is to reduce hyperinflation and gas trapping by creating extra-anatomical airways bypassing expiratory flow limitation utilising stents to maintain patency of the airway created. LVRCs aim to improve these parameters by improving small airway patency by applying traction forces across lung parenchyma thus reducing expiratory airway collapse.

Bronchoscopic interventions can be broadly divided into:

(I) Reversible airway interventions. These include endobronchial valves; LVRCs and transbronchial stents. These may potentially be retrieved if complications occur;

(II) Irreversible interventions inciting an inflammatory/ fibrotic response or irreversibly plugging distal airways. These include bronchoscopic thermal vapour ablation (BTVA) and biological LVR (BioLVR).

Of these interventions the largest body of evidence is currently available for endobronchial valves, although as we will see collateral ventilation has limited its overall efficacy and translation to clinical practice. The current focus is on identifying and selecting patients without collateral ventilation for whom the technique may be of benefit. BTVA and LVRCs show promise although large scale randomised trials required to support their widespread use are currently pending or not available. The majority of these techniques rely on analysis of HRCT images via software packages to facilitate precise targeting of the most diseased lung parenchyma.

Endobronchial valves

Endobronchial valves allow unidirectional airflow. When sited in bronchi leading to hyper-expanded, emphysematous

lung parenchyma, air is permitted to escape on expiration with no corresponding inspiratory flow. Lung distal to the stent, assuming no collateral ventilation, will collapse and become atelectatic. Resultant reduction in lung volume should have the same physiological effect to surgical LVR. At present two valve products are marketed (Zephr™ and IBV); despite differences in valve design the physiological principles for action are similar.

Results of the initial large randomised trial (VENT study) (23) were not as encouraging as the preliminary studies (24). A total of 321 patients were randomised to Zephr™ endobronchial valve placement or best medical care with a 2:1 ratio. A sham procedure was not undertaken in this study. Patients all had severe airflow obstruction and radiologically heterogeneous emphysema quantified on HRCT chest. Although the study showed statistically significant improvement in the primary outcomes at 6 months (FEV$_1$ 4.3% increase; 6MWT 9 meters improvement) the magnitude of these changes was deemed unlikely to be clinically meaningful (25). Pre-defined major complications were seen in 4.2% of patients undergoing valve therapy. Although not pre-defined as major complications, 7.9% and 5.6% of patients experienced an exacerbation of COPD requiring hospitalisation or haemoptysis respectively.

The European arm of the VENT trial (n=171) was commenced to support slow recruitment in the American study (26). Target recruitment was eventually achieved hence the European cohort being reported separately. Study design was similar to the American arm. When looking at the study population as a whole, a statistically significant improvement at 6 months was seen in only cycle ergometry (5 watts mean improvement compared to controls; P<0.05) and SGRQ. The change in SGRQ (5 points) was again below the threshold considered clinically meaningful. The reported focus on this second paper from the VENT group was the effect of collateral ventilation and complete lobar isolation. Subjects in the treatment arm underwent further evaluation with HRCT 6 months post procedure to assess degree of airway occlusion and volume reduction of the targeted lobe. Forty-four subjects in the treatment group of 111 had a complete fissure suggesting the absence of collateral ventilation. A complete fissure conferred reduction in lobar volume by 55% compared with 13% where the fissure was incomplete. Lobar isolation was seen in 48% of patients at 6 months (assessed by HRCT) indicating most patients continued to ventilate the targeted lobe despite the procedure. Combining these two variables

(no collateral ventilation; successful technical isolation) yielded the most encouraging results. Improvements in FEV$_1$, 6MWT and St George's questionnaire were all clinically and statistically significant in this instance.

Ninane *et al.* tested IBV valves in a sham procedure controlled study (n=73) (27). Upper lobes were targeted although the study design was such that complete lobar occlusion was deliberately avoided to prevent lobar atelectasis which the study author hypothesised may cause adverse events. The primary outcome was proportion of patients responding to treatment by reaching a composite endpoint of change in SGRQ and lobar volume (defined as a 4-point increase in SRGQ, reduction in target lobe volume and 7.5% increase in lower lobe volume at HRCT assessment at 3 months). Although significantly more patients in the treatment group responded (8/33 *vs.* 0/35, P=0.002), the majority of patients did not respond to the treatment. The study design and avoidance of lobar atelectasis may account for the low proportion of responders.

The success of endobronchial valves is therefore highly dependent on lobar isolation and collateral ventilation which, as described above, occurs in a significant number of patients. Further techniques have been developed to assess CV (28). The Chartis system allows the targeted lobe to be occluded with an endobronchial balloon with measurement of expiratory airflow and pressure distal to the occlusion. Presence of flow distal to the balloon occlusion is suggestive of CV. This system can be used to determine which patients are more likely to respond to the insertion of endobronchial valves based on the measurement of CV (29). In this cohort of 96 patients undergoing endobronchial valve insertion 35% were assessed as having collateral ventilation present at bronchoscopy utilising Chartis. The system predicted response to insertion of endobronchial valves. Absence of CV conferred mean lobar volume reduction of 751 mLs compared to 98 mLs where CV was present (P<0.0001). These figures are clinically relevant as volume reduction in target lobe has been correlated with reduction in BODE index (body mass index, obstruction, dyspnoea and exercise tolerance) at 6 months (30).

The main limitation for using Chartis to assess collateral ventilation and predict which patients stand to benefit is the requirement for bronchoscopy. Patients with CV found at bronchoscopy precluding (or predicting poor response) to endobronchial valve placement would have undergone a procedure with limited potential for therapeutic benefit. At present this must be factored into the risk benefit analysis.

IP3 identifies diseased region for treatment

Bronchoscope is positioned into airway of diseased region
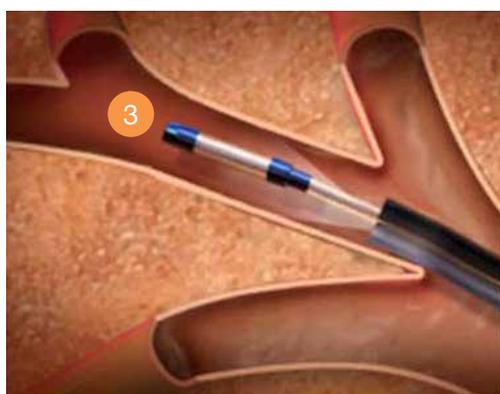
Vapor catheter placed via bronchoscope in airway

Vapor delivered for 3 to 10 seconds based on mass of region

Figure 1 Technical aspects of BTVA-courtesy of uptake medical corporation. BTVA, bronchoscopic thermal vapour ablation.

Limiting Chartis assessment for CV to patients with complete fissures identified at radiology may improve the yield of bronchoscopic assessment identifying subject most likely to benefit from valve therapy. A trial addressing this question is currently recruiting (31). An alternative strategy might be to use an alternative irreversible CV independent technique in patients where CV is identified as described below.

Bronchoscopic thermal vapour ablation (BTVA)

This technique causes a thermal injury via heated water vapour to emphysematous lung to induce an inflammatory response. The resulting atelectasis and fibrosis reduces the volume within the targeted lung segment potentially conferring similar physiological effect to conventional LVRS. Unlike endobronchial valves, the technique is not dependent on collateral ventilation.

Snell *et al.* published a case series of 44 patients undergoing unilateral BTVA (32). Patients with severe airway obstruction (FEV_1 15-45% predicted) were included if heterogeneous upper lobe emphysema was present as defined by lower lobe: upper lobe tissue to air ratios of >1.2 on baseline HRCT scan. This scan was used to plan treatment location and dose using predefined algorithms. In the above trial the 10 cal/gram dose of steam vapour was directed to the most diseased lung parenchyma. The targeted segments are intubated using a catheter directed through the bronchoscope working channel. A balloon is then fed over the guide catheter and inflated to protect the non-treated lung and airways prior to the predefined vapour dose being delivered (*Figure 1*). Follow-up to 6 months demonstrated encouraging results. Significant volume loss was seen in the targeted lobe (mean reduction 715 mL; P<0.001), FEV_1 improved (141 mLs, P<0.001) as did 6MWT distance (46.5 metres, P<0.001). Symptomatic improvement was reported although these improvements must be interpreted with caution given the absence of a control group.
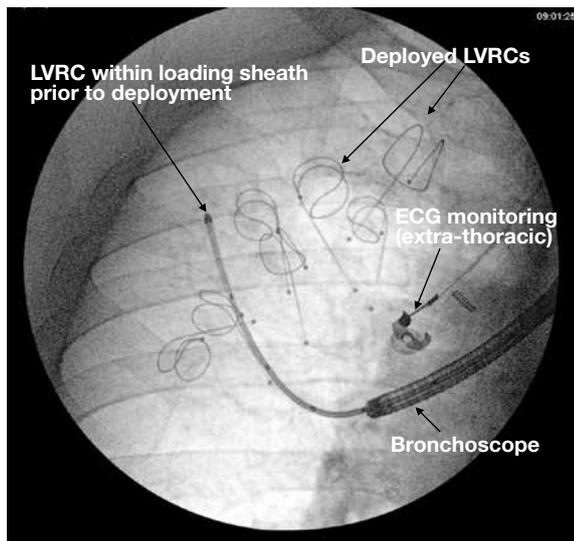
Figure 2 Fluoroscopic appearance of endobronchial coils at bronchoscopy. Seven coils have been sited with the 8th coil remaining within the guide sheath just prior to deployment. Courtesy of PneumoRx Inc.

Given the mechanism of LVR-thermally induced lung injury and inflammation-it is unsurprising that respiratory complications were reported. A total of 25 of 29 adverse events were of a respiratory aetiology (43% of patients). COPD exacerbations and pneumonia were recorded in the 3 months following the procedure. A single death due to 'end stage COPD' was reported at 67 days. Follow-up analysis demonstrates that patients who experienced symptoms attributable to the localised inflammatory response derived greater benefit from the procedure in terms of volume reduction (33). A randomised phase III "Step-Up" trial is currently underway (34), recruiting 69 patients with heterogeneous bilateral upper lobe emphysema randomised 2:1 to either sequential bilateral upper lobe BTVA 3 months apart or best medical therapy. The treatment will clarify the role of this therapy and provide important safety data.

Lung volume reduction coils (LVRCs)

By applying traction forces to lung parenchyma, LVRCs aim to improve hyperinflation and gas trapping by reducing dynamic airway collapse (22). The mechanism of action is again independent of CV and could be applied to emphysema that is homogeneous or heterogeneous (in contrast to BTVA where heterogeneous disease is currently being targeted). The early published data shows promise

with larger studies underway (35,36). The technique involves catheterising target lung segments with a guide wire to a distance 3.5 mm to the pleural edge (*Figure 2*). The coil sits within a loading sheath, straightening it prior to deployment. As the sheath and guide wire are withdrawn the LVRC reverts to its prior coiled shape applying traction to the surrounding lung parenchyma. Dynamic expiratory small airway collapse is reduced by application of radial traction thus improving gas trapping and hyperinflation. Up to ten LVRCs can be sited during a procedure initially unilaterally with further scope for a contra-lateral procedure at a later date if tolerated.

The most comprehensive evaluation of LVRCs was published as the RESET trial (35). Forty-seven patients were randomised to either LVRCs or usual care (1:1) with follow up to 90 days. Inclusion criterion included severe airflow obstruction (FEV_1 <45%), emphysema on HRCT, TLC >100% and dyspnoea (MMRC score >2). Primary outcome was SGRQ with secondary outcomes including 6MWT, FEV_1 and MMRC dyspnoea score. Although baseline characteristics were not matched, clinically and statistically meaningful improvements were seen in SGRQ (8.36 between group improvement P=0.04) and 6MWT distance (63.55 metre between group improvement, P<0.001). No improvement in TLC was seen at 90 days. Further studies are required and are currently recruiting to further evaluate this technique in larger cohorts of patients (35).

Biologic lung volume reduction (Bio-LVR)

The principle of bio-LVR is similar to that of bronchoscopic thermal ablation. A fibrinogen based biopharmaceutical suspension containing thrombin polymerises when instilled into targeted airways (20). The resulting biodegradable matrix induces a localised inflammatory response inducing fibrosis and collapse of the targeted segment. Nonrandomised phase II studies evaluating optimal dose and safety demonstrated significantly improved FEV_1, RV/TLC ratio and RV in 22 patients undergoing higher dose (37). The treatment was associated with transient fevers, leukocytosis and COPD exacerbations. Despite promise, phase III trials were not further pursued, presumably due to the development of the alternative preparation Aeriseal® by the study sponsor.

In contrast to bioLVR, the Aeriseal® preparation aims to induce LVR acting at bronchiolar and alveolar levels by sealing airways inducing absorption atelectasis thus leading to reduction in lung volume. The proposed mechanism may also obscure collateral ventilation pathways. Non-randomised

case series have examined the safety of this intervention (38). Magnussen *et al.*'s later case series is the most comprehensive evaluation of the intervention (39). Fifty-four patients with Global Initiative for Obstructive Lung Disease (GOLD) stage III or IV COPD, gas trapping RV >135% (mean 242%) and hyperinflation were evaluated with HRCT to assess for upper lobe emphysema. All included patient were treated with Aeriseal at 2-4 subsegemental sites and followed to 12 weeks. The authors further divided the cohort into patients for whom data with regard to fissure integrity was available. In this subset of 28 patients TLC reduced by 214 and 261 mLs in patients with and without complete fissures respectively. There was no significant difference between the magnitude of change when assessing for the presence of radiologically intact fissures suggesting the treatment is independent of CV. Six-minute walk distance improved by a mean of 31.9 metres with 31% of patients achieving a clinically meaningful improvement of 54 metres. Despite promise the phase III trial was terminated by the study sponsor in November 2013 prior to publication (40). At present the only registered trial recruiting is a phase II study evaluating the role of autologous blood as a biological irritant to induce LVR (41). Given the absence of phase III trials actively recruiting, it is unlikely that biological methods of LVR will implemented into routine clinical practise in the near future.

Endobronchial and extra-pulmonary bypass procedures

Airway bypass procedures have been proposed to reduce gas trapping by directly relieving trapped air in emphysematous lung by creating extra-anatomical airways. Bronchoscopic fenestrations between large airways and diseased lung parenchyma are created to improve expiratory flow. Drug eluting stents are then sited in an attempt to maintain ongoing patency of the novel tracts. The procedure was proposed for those patients with homogenous (diffuse) emphysema. Unfortunately the large (n=315), randomised, sham procedure controlled study evaluating the technique showed disappointing results (42). Improved FVC immediately post procedure was not sustained past 1 month. There was no difference in MMRC dyspnoea scale. Adverse events occurred at higher frequency in the treatment group although serious adverse events were rare. The authors hypothesised that lack of sustained response likely related to occlusion of the stent with mucus or granulation tissue. At present there is no role for the technique-whether changes to stent design might improve long term efficacy remains unevaluated.

An alternative extra-anatomical approach has been suggested and is in early developmental stages (43,44). Expiratory flow rates may be augmented by surgically creating a fistula between the diseased hyper-inflated lung parenchyma and the chest wall thus reducing hyperinflation. The larger calibre bypass airway created is likely to be less prone to occlusion than transbronchial airway stents. The initial case series (six patients) utilised an improvised endotracheal tube to maintain airway patency. Custom designed pneumonectomy catheters-the 'portaero pneumostoma' have subsequently been developed and are under evaluation (45). The risk benefit profile for this method of LVR will require careful evaluation (*Figure 3*).

Lung transplantation

Indications for lung transplantation in COPD

Despite significant symptoms and functional limitation patients with advanced COPD have survival which is variable due, generally, to slow chronic disease progression over years. Median survival of patients with GOLD stage III and IV disease is 6 years (46). After transplantation, patients with COPD have median survival of 5.4 years with 30% of transplanted patients surviving to 10 years (47). Given that goals of transplantation are improvement of symptoms and survival, patient selection and identification of subgroups of patients with poor prognosis is critical. The presence of severe airway obstruction alone is insufficient to predict who might benefit. Whether lung transplantation should be offered to palliate symptoms without improvement in survival benefit is contentious, especially given limited availability of donor organs (48). In general terms, lung transplantation is indicated where predicted survival is less than 2 years in patients with NYHA III or IV symptoms and associated poor quality of life. The presence of absolute or relative contraindications must be considered and factored into clinical decision making when proceeding to transplant (*Tables 1,2*) (48).

Patients should ideally be referred to a transplant centre before they are established in the "transplant window"- the time period for which the patient is likely to confer benefit from transplantation prior to becoming too frail to undertake the peri operative rigours and recovery after transplantation. This allows adequate time for assessment, consideration of alternative options (i.e., LVRS as discussed above) and addressing reversible relative contraindications or issues that may impact on the transplant process. Factors
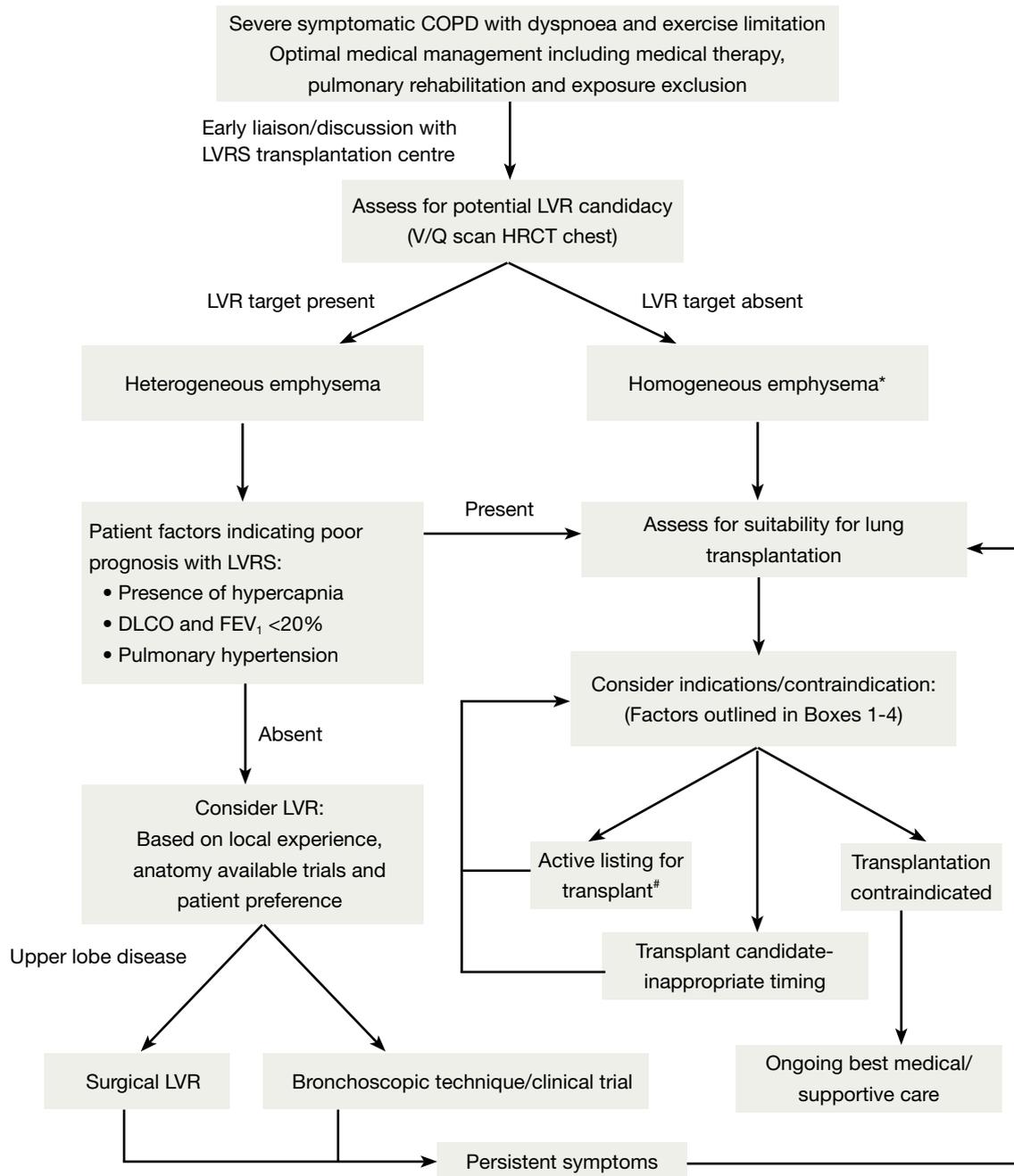
Figure 3 Suggested pathway for management of advanced COPD. *, in selected centres trial may be available for patient with homogenous disease. #, patient active on transplant waiting list require ongoing evaluation. COPD, chronic obstructive pulmonary disease; LVRS, lung volume reduction surgery; LVR, lung volume reduction.

which should prompt referral to a transplant unit in patients considered appropriate are outlined in *Table 3*.

Acute COPD exacerbations with associated hypercapnia (PCO_2 >50 mmHg) confer a poorer prognosis with associated 2-year median survival of 49% (49). This study was performed prior to NIV becoming routine for exacerbations associated with hypercapnia. A total of 89% of the study cohort survived the index admission which suggests that such exacerbations may be a marker for progressive disease and death.

Table 1 Absolute contraindication to lung transplant
Malignancy within last 2 years
Advanced untreatable disease of another major organ system
Non-curable extra-pulmonary infection
Chest wall deformity
Non-adherence with existing medical therapy
Lack of reliable social support
Substance addiction or abuse

Table 2 Relative contraindications to lung transplant
Age >65
Critically unwell (i.e., mechanical ventilation or extra-corporeal membrane oxygenation)
Limited functional status
Obesity (BMI ≥30)
Osteoporosis (particular caution with history of low impact fractures)
Colonization with resistant organisms
Presence of medical conditions which may impact on post transplant course

Table 3 Factors indicating deterioration which should prompt referral to transplant centre
Progressive disease despite optimisation of pharmacotherapy, pulmonary rehabilitation and exposure cessation
FEV_1 <30% predicted
BODE index >5
No suitable target for LVRS
Acute exacerbation with associated hypercapnia
Pulmonary hypertension despite oxygen therapy
LVRS, lung volume reduction surgery.

Scoring systems may also have a role in identifying patients with poor prognosis (50). The BODE score further uses body mass index (B), degree of obstruction (O), dyspnoea (D)-MMRC dyspnoea scale, and exercise capacity (E)-6 minute walk test (6MWT) to stratify which patients have poorer prognosis. Scores of 7-10 confer median survival of 3 years indicating patients are symptomatic, functionally limited and are likely to have a survival benefit from transplantation. The NETT trial also identified a subgroup of patients with poor prognosis. Subjects who did not undergo LVRS (control group) with low FEV_1 (<20%), and either low DLCO (<20%) or homogenous emphysema survived for a median of 3 years although this was significantly better than similar patients undergoing

LVRS. Patients with refractory pulmonary hypertension despite oxygen therapy should also be considered given high waiting list mortality (51).

In appropriately selected patients, lung transplant is associated with significant improvements in quality of life and exercise capacity (52,53). Despite COPD being the leading indication for lung transplantation accounting for 33.5% of procedures worldwide, it remains a highly limited resource. The 12,602 procedures have been performed for this indication worldwide between 1995 and 2012. In the United States the lung allocation score (LAS) was introduced to objective prioritise patients on the transplant waiting lists at highest risk of mortality (54). Whilst this intervention has improved waiting time and mortality for patients with idiopathic pulmonary fibrosis, conversely COPD patients can expect to wait longer for lung allocation (55). The main barriers limiting transplantation to a minority of patients are donor organ availability and cost. Increasing the numbers of organs available for transplant can be achieved either by:

(I) Increasing the percentage of eligible donors identified or consenting to transplant. Large variation in organ donation rates worldwide reflect legal, cultural and organisation differences and has been comprehensively reviewed elsewhere (56);

(II) Changing retrieval techniques and practises. The emerging practise of donation after circulatory death (DCD), in addition to the more conventional brainstem death donors;

(III) Improving utilisation rates of organs offered for transplantation using novel technologies such as *ex vivo* lung perfusion (EVLP).

Donation after circulatory death (DCD)

DCD is not a new concept, reintroduced clinically in 1995 (57), but not widely practised due to concerns about prolonged warm ischaemic time and inferior organ assessment opportunity. Donation after brain stem death (DBD) has been the traditional source of donor lungs. Over the last decade, DCD has emerged as a significant pool of donor organs enabling an increase in transplant volume. Since the 2006 introduction of lung DCD programmes in Australia, 12.4% of organs have been acquired from DCD (58). By 2010 this represented an extra 28% of donors being utilised. The Maastrict classification established in 1995 describes the different circumstances whereby DCD organ donors may be procured (59). Briefly, Maastrict categories I and II refer to uncontrolled deaths in patients deceased

Table 4 Conventional criterion for acceptance of lung donors

Age <55
PaO_2 >300 mmHg (5 mmHg PEEP FiO_2 100%)
Clear chest X-ray
Less than 20 pack years smoking
Absence of chest trauma
Absence of prior thoracic surgery
Absence of aspiration or sepsis

on arrival at hospital or with unsuccessful resuscitations attempts respectively. Category III-death after controlled withdrawal of supportive treatment (usually in an intensive care unit) describes the majority of DCDs in Australia, USA and Europe (excluding France and Spain where category II donors are more common) (60). Categories IV and V refer to circulatory collapse after brainstem death and inpatient cardiac arrests respectively-these are not common modes of organ procurement.

Clinical outcomes of patients receiving DCD lungs are comparable to that of conventional lung donors (58,61,62). The Australian DCD collaborative is the largest reported series of exclusively Maastrict III donors (58). Short and long term DCD outcomes are similar to that of DBD patients over the same time period. Among 72 patients receiving DCD lungs, 1 and 5 year survival was reported at 97% and 90% respectively (90% and 60% for 503 patients undergoing DBD during the same time period). Incidence of primary graft dysfunction (PGD) and bronchiolitis obliterans syndrome was similar between groups. This supports the notion that group III DCD donors which otherwise meet conventional acceptance criterion (*Table 4*) should not be considered 'marginal'. This is in contrast to practise in other centres where EVLP has been routinely employed for all DCD lungs (63).

Ex vivo lung perfusion (EVLP)

Lung transplantation is dependent on the availability of organs from suitable donors. Respiratory complications in potential lung donors contribute to a low proportion of organs proceeding to transplantation. Common donor mechanisms of death-chest trauma, aspiration, ventilator associated pneumonia, barotrauma and systemic inflammatory response syndrome all impact on organ utility. Transplant physicians exercise caution when assessing potential donor lungs to minimise the risk of

morbidity and mortality from PGD-a condition associated with inferior short and long term outcomes (64). It is seen more frequently in patients where there is deviation from traditional donor acceptance criterion (*Table 4*) (65). These parameters will minimise the risk of PGD but lead to a low proportion of potential donors converting to transplant. Of organs offered for transplant a low proportion—15% to 20%—are utilised (66). Strategies to safely increase the number of "marginal" donors-those organs with clinical features/parameters deviating from traditional acceptance-will have an impact on numbers of patients able to undergo transplantation. Reported results from some larger transplant centres suggest those traditional acceptance criterions are overly stringent (67) with transplantation being safely undertaken where the donor does not fully adhere to this criteria. Recognition that these criteria are not absolute may be contributing to recovery of a higher proportion of organs (68). EVLP is a further tool that has potential to further improve this trend.

EVLP is used in the assessment and reconditioning of donor lungs. The technique was first introduced by Steen *et al.* in 2001 for graft assessment after Maastrict II DCD (69). The Toronto group recognised the potential of the technique for addressing donor respiratory complications. Refinement to the process means that lungs previously discarded can be reconditioned, re-assessed and if suitable transplanted (70). Potential indications for the use of EVLP although not standardised reflect deviation from traditional acceptance criterion (63,71):

(I) PaO_2/ FiO_2 <300 mmHg with PEEP 5 cm H_2O;
(II) Infiltrates on CXR (pulmonary oedema/pneumonic consolidation);
(III) Poor lung compliance or PEEP dependent donor lungs;
(IV) Questionable aspiration history;
(V) Logistical difficulties resulting in anticipated prolonged cold ischaemic time.

As outlined above, procurement of DCD donors has been used as an indication for EVLP (63) although other centres have demonstrates satisfactory DCD outcomes without this additional assessment (58). Controversy exists with regard to EVLP in where it should be employed. As mentioned above, a proportion of marginal donors can be utilised without EVLP assessment without compromising outcomes (67); given this more work is required to redefine the boundaries of donor conventional donor acceptability. Such studies may define where marginal lungs could be utilised without EVLP-without this information there is a
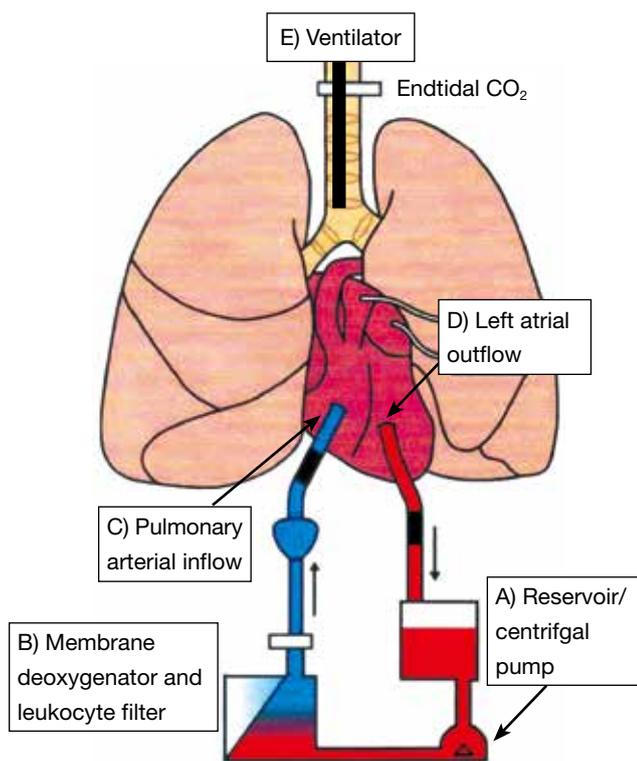
Figure 4 Summary diagram of the EVLP circuit: (A) steen solution™ and blood are circulated via a centrifugal pump; (B) a membrane de-oxygenator allows assumption and regulation of gas pressures equivalent to mixed venous blood. The leukocyte filter minimised leukocyte mediated tissue injury; (C) the pulmonary artery is cannulated. Pulmonary arterial pressure is monitored and flow rate regulated to prevent oedema; (D) left atrial outflow is sampled allowing graft assessment; (E) gentle ventilation commences at a temperature of 32 ℃ full ventilation at 37 ℃ prior to graft assessment. EVLP, ex vivo lung perfusion.

risk that the technique could become standard of care prior to these limits being clarified.

The EVLP circuit consists of a sterile chamber housing the donor lungs, centrifugal pump circulating the perfusate, leucocyte filter and membrane de-oxygenator (*Figure 4*). Two differing protocols are currently used and referred to as Lund protocol (72) and Toronto protocol (64), although the general principles are common to the two methods. The perfusate provides above normal oncotic pressure and inhibits endothelial leucocyte interaction, generation of reactive oxygen species and thrombogenesis. Gradual warming of the solution occurs to 37 ℃ allowing restoration of cellular metabolic pathways permitting return to physiological

conditions at normothermia. Antibiotics can be administered and interstitial oedema improved via hyperosmolar perfusate mediated fluid shifts. Lungs are connected at an initial perfusate temperature of 15 ℃; at a temperature of 32 ℃ gentle ventilation is commenced with recruitment manoeuvres enabling re-expansion of lobar or segmental collapse. Bronchoscopy may also be performed to assess for and remove secretions from the tracheo-bronchial tree.

Initial data suggests that outcomes with EVLP are similar to conventional lung transplants (63,71,73,74). The HELP study prospectively assessed the role of EVLP in a non-randomised clinic trial (63). A total of 306 donor offers were assessed; 111 donors proceeded directly to transplant whilst 23 underwent EVLP management having met pre-defined high risk criterion. Of these EVLP conditioned donor lungs 20 were successfully transplanted (3 EVLP assessments were deemed unsatisfactory for transplant). No significant differences in PGD or mortality were seen to 30 days compared with control subjects undergoing standard transplantation procedure. The same group report later reported EVLP conditioned lungs accounting for 20% of their transplant activity-significant given these organs would otherwise not be utilised (71). Larger multicentre trials are currently underway aiming to confirm these preliminary findings—that EVLP can be safely used to increase donor number (75).

Conclusions

Despite the high prevalence of advanced COPD, current therapeutic options in medically optimised patients are available to a minority. For LVRS, the NETT trial showed that patient selection is critical to outcome and limits the availability to those patients with heterogeneous upper lobe disease. The procedure comes with a risk of morbidity and mortality which has led to the development of less invasive methods of LVR. With time, these may improve accessibility for patients. At present the evidence is insufficient to firmly recommend bronchoscopic LVR methods. Endobronchial valves, the most comprehensively evaluated technique, require lobar isolation and CV to be absent. Work is currently underway to further develop patient selection pathways to prospectively predict who may benefit. Non CV dependent techniques (BTVA and LVRCs) are promising, but require larger randomised trials to confirm efficacy and their safety. In patients for whom LVR is not an option due to absence of an LVR target or contraindications, lung transplantation may be considered.

Its widespread application is limited by cost, rigorous selection criterion and organ availability. Work is underway to improve the accessibility of this limited resource. EVLP is an emerging technique which may assist with this by increasing the proportion of potential donors utilised with early data suggesting such transplants comparable to conventional procedures. Further work is required to define indications for EVLP and conversely circumstances where conventional organ acceptance criterion can be confidently extended.

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Anxiety and depression—Important psychological comorbidities of COPD

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Abstract: Anxiety and depression are common and important comorbidities in patients with chronic obstructive pulmonary disease (COPD). The pathophysiology of these psychological comorbidities in COPD is complex and possibly explained by common risk factors, response to symptomatology and biochemical alterations. The presence of anxiety and/or depression in COPD patients is associated with increased mortality, exacerbation rates, length of hospital stay, and decreased quality of life and functional status. There is currently no consensus on the most appropriate approach to screening for anxiety and depression in COPD. Treatment options include psychological [relaxation, cognitive behavioural therapy (CBT), self-management] and pharmacological interventions. Although there is some evidence to support these treatments in COPD, the data are limited and mainly comprised by small studies. Pulmonary rehabilitation improves anxiety and depression, and conversely these conditions impact rehabilitation completion rates. Additional high quality studies are urgently required to optimise screening and effective treatment of anxiety and depression in patients with COPD, to enhance complex chronic disease management for these patients.

Keywords: Chronic obstructive pulmonary disease (COPD); anxiety; depression; comorbidities; diagnosis; therapy

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease that has significant extrapulmonary effects that may impact the severity of symptoms in individual patients. COPD is a highly prevalent disease worldwide. The prevalence is variable between countries, but overall there is a prevalence rate of around 10% in individuals aged 40 and above (1). In developed countries, COPD is responsible for approximately 4% of all deaths and is the only major condition for which the burden of disease continues to increase, currently being 5th overall in underlying cause of death and 3rd for burden of disease (2).

Anxiety and depression are well-recognized major comorbidities in COPD (3), and consequently there has been a surge in clinical and research interest in reducing the negative impact of these important comorbidities in patients with COPD (4). This review provides an overview of the pathophysiology associated with anxiety and depression in COPD patients, the prevalence and impact of these comorbid conditions, and the strategies for their diagnosis and treatment. Areas of need for future research are also highlighted.

Prevalence of anxiety and depression as comorbidities in COPD

Like other major chronic diseases, COPD has a significant

impact on psychological well-being of people affected. Patients with COPD have a higher prevalence of depression and anxiety than the general population (5) and COPD patients have relative risk of 1.69 of developing depression (6). The rates of both anxiety and depression may even be more prevalent among COPD sufferers compared with other chronic diseases (7).

The reported prevalences of each condition are quite varied, depending on the population surveyed and the tools used to assess depression and anxiety. For patients with stable COPD in primary care settings or respiratory clinics, the prevalence of depression varies widely from 10% to 57% (5,8), and for anxiety, prevalence ranges from 7% to 50% (5,9).

Risk factors for increased rates of depression include living alone (10) and gender. Females have a higher rate of both anxiety and depression (11-13), and rates of depression are more strongly correlated with severity of dyspnoea as compared with males (12). Increasing severity of COPD is associated with higher rates of depression and anxiety (14,15); for example, in patients requiring long-term oxygen, 57% were found to have depressive symptoms and 18% had depression classified as severe (16). End-stage COPD patients undergoing palliative care also have high rates of anxiety and depression (7).

Other important risk factors are patients that have been hospitalized for an exacerbation of COPD or recovering from an exacerbation (17,18), severity of respiratory symptoms especially dyspnoea (19), living alone, and severe impairment of physical functioning (9,10).

Pathophysiological mechanisms for anxiety and depression in COPD

The aetiology of the association between depression and COPD is not fully understood; however the relationship is complex and interactive. The most important risk factor for COPD is smoking. Smoking and depression have a bidirectional interaction. Depressed individuals are more likely to smoke (20), display higher risk to commence smoking (21,22), and find smoking cessation more difficult (20,23). Conversely, smokers are more likely to be depressed (24), which could be caused by activation of nicotinic acetylcholine receptors (25), or direct inflammatory effects of smoking (26).

Although smoking could have some part to play as a causative factor for depression, depression is still more prevalent among COPD patients than smokers without

COPD (13). A possible mechanism could be related with 'overspill' of local lung inflammation in the circulation (26,27). It has been speculated that systemic inflammation may play a role in the presence of depression (28). Although there are difficulties in quantification of inflammatory markers in the 'overspill' theory (26), sTNFR-1 has shown a strong association with rates of depression in COPD patients (27), while TNF-α has shown conflicting results (28,29). It is not clear if the presence of systemic inflammation has a causative association with depression or that it is a marker a specific COPD phenotype; such as frequent exacerbators (27).

Hypoxia is an additional factor that may play a role in the development of depression in COPD. Low arterial oxygen saturation has been shown to be associated with periventricular white matter lesions (30), which are present in patients with depression (31). However, the significance of these findings is contentious since the localization of subcortical hyperintensity in depressed patients has been found to be variable due different imaging technologies, lesion definition and measurement techniques (31,32).

Although smoking, inflammation and hypoxia have potential impact on the prevalence of depression in COPD, the strongest predictors of depression among patients with COPD are their severity of symptoms and reported quality of life (13). Functional limitations have been similarly shown to mediate depression in other disorders such as arthritis and heart failure (33). The amount of perceived instrumental support (the need of assistance for activities of daily living) among COPD patients has also been shown to be correlated with depression (34).

Several theories have been proposed to explain the overlap of anxiety and panic attack symptoms with COPD (35). Hyperventilation is defined as the exaggerated breathing in excess of metabolic need, causing lowering pCO_2 and causing respiratory alkalosis (36,37). This pattern of breathing can cause dyspnoea in healthy individuals and consequently panic attacks in those predisposed patients (37).

In panic disorder patients it is possible to evoke symptoms of dyspnoea and chest pain when infusing lactate or inhaling excessive CO_2 (37). These findings are the basis of the carbon dioxide hyperventilation model (35). Areas of the brain with intrinsic CO_2/H^+-sensitive neurons such as the ventrolateral surface of the medulla and locus coeruleus are involved in ventilation, but also play role in panic behaviours. The activation of these areas may concomitantly activate a defensive behavior and precipitate a panic attack (37).

Another important theory is the cognitive behavior

model which is based on the principle that normal bodily sensations are misinterpreted by patients with panic disorder and can consequently cause a panic attack (35). This misinterpretation may be associated with a behavioural sensitization event (trauma), since 20-30% of healthy panic disorder patients had a near-drowning or suffocating past experience (37). COPD patients are at greater risk of a traumatic event caused by an exacerbation, which may lead to an increased risk of developing panic disorders.

The pathophysiology of anxiety and depression among COPD patient is complex and poorly understood. Patients with depression and anxiety are at higher risk of developing COPD due to smoking. Likewise the physical, emotional and social impact of COPD is correlated with development depression and anxiety. This complex interaction between COPD and mental health diseases may cause a self-perpetuating cycle that has a severe impact upon a patient's well-being.

Impact of depression and anxiety on COPD

Depression and anxiety have considerable impact on patients with COPD, in terms of associations with mortality, exacerbations and quality of life.

Effect on mortality

Among COPD patients, depressive symptoms are associated with increased mortality among hospitalized (18,38) and community patients (15,39-41). Some studies of COPD patients have shown an association of anxiety with increased mortality (18,38,42), whereas others have failed to show any association (41). A recent meta-analysis demonstrated that in COPD patients, comorbid depression and anxiety were associated with increased risk of mortality with relative risks of 2.29 and 1.27 respectively (6).

Importantly, a prospective study by Divo and colleagues, from the BODE cohort, has demonstrated that anxiety among female COPD patients was associated with a significant increase in mortality, with a hazard ratio of 13.76 which was more than the risk conferred by coronary heart disease, heart failure, or lung cancer (43). The potential causes of this increased mortality with anxiety are probably multifactorial. One factor is treatment compliance; for example, patients with depression are more likely to not complete rehabilitation (44,45). A meta-analysis has showed that patients with depression and anxiety symptoms are 3 times more likely to be non-adherent to their prescribed

medications (46). Alternatively, anxiety may be secondary to the severity of the underlying COPD, and could therefore be a clinical marker of disease severity and risk of death.

Effect on exacerbations

Among COPD patients, exacerbations contribute significantly to morbidity and mortality (47). A systematic review of 20 studies has shown that depression and anxiety increases the risk of hospitalization for COPD patients (48). A meta-analysis by Laurin *et al.* showed that the relative risk of in-hospital treated COPD exacerbation was 1.12 for depression and 1.18 for comorbid depression and anxiety (49). Anxiety and depression symptoms were also associated with increased length of stay in hospital for COPD exacerbations (18,50,51).

There are multiple possible links between depression and anxiety, and increased rates of COPD exacerbation. The impact of symptoms of depression and anxiety could place patients at risk due to non-adherence with treatment (46,52), and suboptimal success with smoking cessation (18,49). Depression could have direct effects by impairing the immune system and consequently predisposing to infections (53) leading to increased frequency of exacerbations. Worsened perception of dyspnoea may lead patient to seek medical attention unnecessarily and increase hospital admissions; patients with anxiety and depression during admission have worse dyspnoea scores despite having less severe physiological parameters (e.g., pH, partial pressure of oxygen and carbon dioxide) (54). The meta-analysis by Laurin *et al.* has shown that patients with anxiety were at greater risk for exacerbations that required treatment in the community, whereas those with depression were at higher risk for exacerbations requiring treatment in hospital (49). This discrepancy could be explained by "early intervention" among anxious patients that could prevent the need for treatment in hospital (49).

Effect on quality of life

The detrimental impact of COPD on quality of life is well-documented (3). Depression and anxiety symptoms also have significant impact on quality of life and functional status in many chronic diseases (55,56). In general, patients with depression and anxiety perceive their health as poorer than the average population (57). Specifically for COPD, the impact of quality of life and functional status is also evident in several studies, independent of the severity of

COPD or related comorbidities (14,56,58-62). A meta-analysis showed that the presence of depression and anxiety among COPD patients was one the strongest correlations with self-reported health status (63). Comorbid depressive symptoms in patients with COPD are associated with persistent smoking, increased symptom burden, poorer physical and social functioning (18), and difficulty in performing daily activities (64). Low self-confidence or self-efficacy is also common, which may lead to worsened ability to cope with chronic disease (49,56).

Depression and anxiety symptoms are associated with increased perception of dyspnoea (54,65,66). The presence of psychological symptoms (mainly depression and to lesser extent anxiety) has an effect on vital exhaustion, defined as a state characterized by fatigue and lack of energy, worsening irritability and feelings of demoralization (67). Fatigue and especially dyspnoea are independently negatively associated with poor health status (63,68).

The impact of depression and anxiety symptoms are not limited to an individual's lung disease. The presence can influence a person's end of life decisions (69) or may have negative impact upon partners and their respective relationships (70).

Diagnosis and screening of depression and anxiety

The gold standard for the diagnosis of depression or anxiety is based in the criteria listed in the DSM-IV and achieved through structured interviews performed by a psychiatrist or a clinical psychologist. As there is a strong positive relationship between self-reported severe symptoms and the existence of a mental disorder (71,72), screening instruments have also been developed, which are less costly, faster and easier to administer. These instruments can also monitor clinical outcomes of mental health treatments (73,74).

Several screening tools have been validated for use in COPD patients. The Geriatric Depression Scale and its 15-item short form (GDS-15) are validated as depression tools (75), and the Hospital Anxiety and Depression Scale (HADS) and the Geriatric Anxiety Inventory (GAI) have been validated for anxiety in COPD patients (76). Anxiety Inventory for Respiratory (AIR) Disease and Brief Assessment Schedule Depression Cards (BASDEC) are two other scales that have been developed exclusively for COPD (9,77).

There are concerns regarding the use of screening instruments due to the risk of false positives caused by the overlap of symptoms (78), and the uncertainty regarding

impact on routine practice (79,80). The Global Initiative for Chronic Obstructive Lung Disease guidelines recommend that new COPD patients should have a detailed medical history including for depression and anxiety (3). However, to date, there is no consensus on the most appropriate screening approach for anxiety and depression (81).

Treatment approaches for anxiety and depression in patients with COPD

Depression and anxiety, when coexisting with COPD, significantly impact quality of life and functional outcomes. In acknowledgement of the biopsychosocial impact of chronic ill health, the World Health Organization has stated that patients with chronic diseases such as COPD should receive integrated care programs which are centered on the patient rather than just the disease (82). Fortunately, interventions targeting these psychological comorbidities are well-established for the general population (83). However psychological care guidelines are less well developed for the specific COPD patient population (84). Where psychological treatments have been used in COPD, these have typically been based on guidelines already in use for depression and anxiety in the wider population (85). Treatments can be divided into psychological [relaxation, cognitive behavioural therapy (CBT), self-management] and pharmacological interventions. Pulmonary rehabilitation, a specific treatment for COPD, also has beneficial effects on anxiety and depression.

Psychological therapies

For patients with a chronic health condition who are also experiencing clinical or sub-threshold depression, the UK's National Institute for Health and Care Excellence (NICE) recommends use of low to high intensity psychosocial interventions depending on the severity of mood symptoms (85). Low intensity interventions may include individual or self-help programs, or online CBT, while high intensity interventions are typically individual or group CBT sessions. These recommendations are based on moderate quality randomized controlled trials and the experience and opinion of the Guideline Development Group (85). While the NICE guideline targets general chronic health presentations, good quality studies are somewhat lacking in COPD-specific populations. Existing studies show mixed results that are difficult to compare, because of factors such as small sample size, varied populations, lack of data on

disease severity and differences in the screening tools used to assess these patients. A recent meta-analysis has described the benefits of the most common psychological interventions—relaxation therapy, CBT and self- management education programs (83).

Relaxation therapy

The aim of relaxation therapy is to promote psychological change through techniques that create a relaxed state. Techniques commonly used range from breathing exercises, hypnoses, meditation, body positioning, sequential muscle relaxation, mild forms of exercise and visualization techniques (86). These methods are used separately or as a element of other psychological treatments or pulmonary rehabilitation (4).

The effectiveness of relaxation-based therapies for COPD was evaluated in a meta-analysis by Devine *et al.*, which showed significant improvements in symptoms of dyspnea and anxiety (87). For patients undergoing a pulmonary rehabilitation program, progressive relaxation techniques administered by tape-recorded classes showed a non-significant improvement in depression and, to a lesser extent, anxiety symptoms at the time of the end of the pulmonary rehabilitation program (88). There have been several smaller studies that have investigated other types of relaxation approaches. One small study using tai chi demonstrated a non-significant improvement of depression, dyspnoea and physical capacity as measured by six minute walk test results (89). A study examining yoga as the intervention showed a significant improvement in six minute walk results and functional performance, non-significant improvement in dyspnoea score and quality of life, but no change in anxiety or depression scores (90). In these types of studies, it is often difficult to determine whether the benefit is due to the physical activity or the relaxation components of the treatment.

Loosely related to relaxation interventions, singing classes have also been used as an intervention in COPD patients. The underlying theory is that singing lessons might improve patient quality of life and/or functional status by offering techniques that address both the sensory component of dyspnoea (e.g., control of respiratory pattern to reduce hyperinflation) and the affective component (e.g., anxiety and low mood around perceived breathlessness) (91). A moderate-sized study employing singing classes showed improvement in anxiety levels and the physical component of a quality of life questionnaire (92). In a further study by the same researchers, the improvements remained after controlling for the incidental beneficial effects of social interaction amongst the participants (93). In regards to these less traditional interventions, there is still a lack of clarity about their applicability, their long-term effectiveness, the active component (physical or psychological), and how they may be incorporated into standard care.

Cognitive behavioural therapy (CBT)

CBT is a type of psychotherapy used in the management of a range of psychiatric disorders. It is based on an information-processing model in which emotional symptoms are thought to be driven by negatively-biased evaluations of the world, the future, or the self (including bodily sensations) (94). Often performed in collaboration between the therapist and the patient, CBT utilizes a number of strategies to correct those biased evaluations and provide skills aimed at controlling their symptoms and consequently improving the management of their illness (4).

The use of CBT has gained traction because of its effectiveness in achieving symptomatic relief for patients with chronic illnesses (56,95). There have been numerous studies of varying quality and sample size that have shown promising results (96-101) (*Table 1*). The studies showed small to moderate improvements in anxiety and depression scores and quality of life; however direct comparison is hampered by the fact that interventions varied in regard to number of sessions, duration of each session and delivery format (group or face to face). The fact that positive impact was demonstrated in most studies, even those with shorter interventions, holds promise for future applicability.

Cost-effectiveness is undoubtedly an important issue, particularly given tightening health budgets, and increasing service imperatives to reduce health care spending. One study has shown that face-to-face CBT is effective and also may be cost neutral when implemented in COPD patients (95). If other less expensive approaches are interchangeable to face-to-face they may be more economically attractive. The use of telephone-based interventions for depression has shown to be just as effective as face-to-face (102-104), and such an approach has also been shown to be beneficial for patients with anxiety and depression associated with other chronic diseases (105,106). A novel alternative approach is the use of an Internet-based intervention, which has been shown to be as effective as face-to-face interventions for depression and anxiety (107-109). CBT-based therapies, particularly tightly manualised therapies for sub-clinical anxiety or depression, may not require a fully trained psychologist for its administration, adding to overall cost effectiveness. A nurse-

Table 1 Major randomized control trials involving cognitive behaviour therapy and COPD for anxiety and/or depression

First author, year	Study design	Patients	Measures	Treatment and comparison	Results
Livermore et al., 2010 (100)	RCT Outpatients Attended pulmonary rehabilitation program Panic attacks and panic disorder were diagnosed with the ADIS-IV	41 patients; 21 CBT, 20 control CBT: Age 73.2±6.4 43% male Control: Age 73.5±8.1 45% male	Primary: Rates of panic attack and anxiety symptoms Panic attacks and panic disorder were diagnosed with the ADIS-IV Secondary: HADS IPBQ SGRQ COPD-related admissions	Intervention: 4 individualized 1 hour sessions and manual (with strategies effective for the prevention and treatment of panic disorder in younger adults) Follow-up: post intervention, 6, 12 and 18 months	ADIS-IV there were significant differences post-intervention and at the 6-, 12- and 18-month No panic attacks in intervention group while no CBT had 35% post intervention tie and 60% at 18 months Significant difference in HADS score at 6-, 12- and 18-month follow-up Significant positive effect on IPBQ No differences in HADS depression scale, SGRQ Significant decrease in hospital admission rate between 6 and 12 months
Hynninen et al., 2010 (97)	RCT Clinically significant anxiety and depression Outpatients Scores >15 BAI and/or >13 on the BDI-II	41 patients; 25 CBT and 26 control 17 participants (33.3%) fulfilled the diagnostic criteria for a mood disorder CBT group: 56% male Age 59.3±7.6 Control group: 42% male Age 62.6±9.9	Primary: BAI BDI-II Secondary: SGRQ PSQI Actigraphy CSQ	Intervention CBT: 7 sessions of 2 hours of group CBT psychology students. Telephone session at 1 and 3 months after Control: telephone contact every 2 weeks for 7 weeks. Call lasted 5-10 minutes Follow-up 8 months	Significant improvement of BAI and BDI-II after treatment and on follow-up Control had no improvement Women had more anxiety and depression, responded more however had more significant anxiety and depression at the end of treatment Treatment was intensive although response was rapid
Kunik et al., 2008 (99)	RCT Outpatients Stable COPD Scores ≥16 BAI and/or >14 on the BDI-II No smoker, low MMSE (<23) or psychiatric disorder	138 patients; 118 to CBT, 120 to control 95% males Education group: 95.8% male Age 66.5±10.4 CBT group 96.6% male Age 66.5±10.1 62.2% had a DSM-IV diagnosis of depression or anxiety	Primary: QoL CRQ SF-36 Secondary: BAI BDI-II 6MWD Use of health services	Intervention: 8× 1 h CBT sessions, group sessions Control: 8× 1 h COPD education sessions Both by same therapist Follow-up: weeks 4 and 8 and months 4, 8 and 12	Both treatments significantly improved QoL with trend favoring CBT group Improvement of SF-36, anxiety and depression (P<0.005) over 8 weeks for both groups No change of 6MWD for either group Follow-up at 8 and 52 weeks showed no change in improvement obtained at end of therapies

Table 1 (*continued*)

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First author, year	Study design	Patients	Measures	Treatment and comparison	Results
de Godoy et al., 2003 (101)	Single blinded RCT COPD attending a pulmonary rehabilitation program	32 patients; 14 to CBT, 16 control. CBT: 85% male Age 62.1±14.9. Control: 62.5% male Age 58.8±11.8	BDI, BAI, 6MWD	Intervention: 12 sessions of psychological sessions: Cognitive therapy and logo therapy techniques. Both had 12 weeks of rehab with: 24 sessions of physical exercise, 24 sessions of physiotherapy, 3 educational sessions	Significant improvement in BAI, BDI and 6MWD. 6MWD improved for both groups, higher improvement in group treated with psychotherapy, although the improvement was not directly related with improvement of anxiety and depression
Kunik et al., 2001 (98)	Single blind RCT Outpatients, from veteran hospital Stable COPD	50 patients; 21 CBT and 29 education group. CBT and education: Age 71.3±5.9 83.1% male	SF-36, GDS, BAI, 6MWD, FEV$_1$	Treatment: same education session lasting 2 hours and 1× 2 h session of group CBT. Control and treatment: 1× 2 hour education session focusing on COPD process, etiology and treatment options. Weekly calls for 6 weeks for both	Statistical Improvement in BAI and GDS and one measure of SF-36 (mental health question). Non statistical improvement of 6MWD
Emery et al., 1998 (96)	RCT Three groups; Exercise, education and stress management (EXESM), Education and stress management (ESM) and waiting list (WL) Outpatients 79 community-based out-patients	79 patients; 30 EXESM, 24 ESM and 25 WL. EXESM: Age 65.4±6.4 50% male. ESM: Age 67.4±5.9 41.6% male. WL: Age 67.4±7.1 48% male	Anxiety and depression: CES-D, The Bradburn Affect-Balance Scale, STAI, SCL-90-R. Health-related quality of life: MHLC, SIP. Cognitive test battery. Pulmonary rehabilitation health knowledge test. Bicycle ergometry testing	EXESM: 10 weeks: 37 exercise sessions, 16 education sessions, 10×1 hour stress management sessions based on CBT and delivered by clinical psychologist. ESM: 16 education sessions, 10× 1 hour stress management sessions based on CBT and delivered by clinical psychologist. WL: Awaiting to be in study. 25 patients. Follow up at 10 weeks	VO$_2$ max = maximal oxygen consumption during bicycle test – improved only with exercise program. However it was not a predictor of any other outcome. Depression improved with exercise and waiting. No improvement with education and psychology. Anxiety reduced more with exercise that without. SIP improved with EXESM and WL. Verbal processing improved only with EXESM. EXESM and ESM improved health knowledge

BAI, Beck Anxiety Inventory; BDI-II, Depression Inventory-II; SGRQ, St George's Respiratory Questionaire; PSQI, Pittsburgh Sleep Quality Index; CSQ, The Client Satisfaction Questionnaire; Disease-specific and generic quality of life (QoL), CRQ, Chronic Respiratory Questionnaire; 6MWD, 6-minute walk distance; CES-D, The Center for Epidemiological Studies-Depression Inventory; STAI, The State-Trait Anxiety Inventory; SCL-90-R, The Hopkins Symptom Checklist; MHLC, The Multidimensional Health Locus of Control; SIP, The Sickness Impact Profile; IPBQ, The Interpretation of Breathing Problems Questionnaire; ADIS-IV, Anxiety Disorder Interview Schedule. Disease-specific and generic quality of life (QoL), Medical Outcomes Survey Short Form-36 (SF-36).

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administered minimal psychological intervention (MPI), based on the principles of CBT and self-management, was used in one study of COPD patients and showed promising results (110).

Not all aspects of CBT therapy may be necessary to produce a therapeutic effect. Purely behavioural interventions can be as effective as CBT for patients with depression (111). They are simpler to administer and theoretically could be used for patients with COPD.

Self-management strategies

Self-management programs aim to improve patient care by providing resources and guiding health behaviour change in ways that empower the individual. This empowerment is thought to increase their ability to carry out medical regimens designed to control their chronic disease, improve well-being and decrease exacerbations (112-114). Many self-management programs incorporate aspects of CBT.

The effects of self-management programs for patients with chronic health conditions are still unclear, and the results have been modest when compared to more specific psychological interventions (83). Jonker *et al.* found improvement in self-efficacy in older people, but no reduction in health care utilization or improvement in quality of life (112). In cardiac patients one study reported a moderate effect of self-management on functional outcomes and depressive symptoms after an acute coronary syndrome (115). For COPD patients, although a review by Kaptein *et al.* reported favourable outcomes for self-management on frequency of hospitalisation, greater exercise tolerance and increased quality of life (116), in the meta-analysis of 29 RCTs by Coventry *et al.*, there was no overall benefit for self-management education alone for anxiety and depression in COPD (83).

A large multicenter randomized trial in COPD patients showed that a self-management intervention reduced exacerbation rates (114). Similarly, a Cochrane review by Effing *et al.* (113) showed a significant and clinically relevant reduction in the number of patients with one or more hospital admissions and a small but significant reduction of dyspnoea scores. Results were inconclusive for anxiety and depression symptoms, doctor and nurse visits, the use of courses of oral corticosteroids and antibiotics, and the use of rescue medication. No effects were seen for ER visits, lung function, exercise capacity, and days lost from work (113). Interestingly, conflicting results for quality of life questionnaires were seen, as a positive trend was seen for the St. Georges Respiratory Questionnaire (SGRQ), but not for SF-36 (113). This last result highlights the need for precision and clarity in the description of the construct being measured (e.g., quality of life) and consistency in the selection of measures. The wide range of measures used across the papers surveyed herein reveals the difficulty in both assessing the effectiveness of interventions within a study, and comparing findings across studies reported in the literature.

Finally, health mentoring is a self-management intervention that uses cognitive behavioural techniques to provide skills to improve self-efficacy and disease management, and to change unhealthy behaviours (117). Nursing-based mentoring has shown conflicting results with one study showing benefit in quality of life for patients with COPD (118), while other studies have failed to show any positive effect on quality of life (119,120) or anxiety and depression symptoms (119). A meta-analysis has shown case management was the least effective intervention for reducing anxiety and depression when compared to CBT, relaxation or self-management intervention (83).

Pharmacotherapy

Pharmacotherapy is a mainstream treatment for anxiety and depression. Although there is some controversy regarding effectiveness, meta-analyses have shown the overall benefit of pharmacotherapy in the treatment of anxiety and depression (121). In standard clinical practice, antidepressants are the main medication used for depression and anxiety. Other less common agents used are benzodiazepines, antipsychotics, anticonvulsants and azapirones (4).

Antidepressants work mainly by increasing synaptic monoamines, dopamine, serotonin and/or noradrenaline. They have similar effectiveness but mainly differ based on type and severity of side effects. The main categories are: selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), noradrenaline reuptake inhibitors, tetracyclic antidepressants, tetracyclic analogues of mianserin [sometimes called noradrenergic and specific serotonergic antidepressants (NaSSA)], tricyclic antidepressants (TCAs), reversible inhibitors of monoamine oxidase A (RIMAs), monoamine oxidase inhibitors (MAOIs) and melatonergic antidepressants [summarised in (122)]. Antidepressants are a moderately effective treatment for depression in healthy individuals, more so in cases of greater severity and in melancholia, but there is less certainty in physically ill patients. Among this population with subthreshold

symptoms of depression (symptoms that are below the DSM-IV criteria for major depression) or mild to moderate depression, NICE guidelines advice that antidepressants should not be routinely prescribed (72).

A recent Cochrane review of antidepressants (mostly SSRI and TCA), for the treatment of depression or depressive symptoms among physically ill patients, demonstrated a significant improvement among patients with depression or milder depressive disorder, and a positive trend for depressive symptoms and other depressive disorders (123). This Cochrane review also observed that there was greater long-term improvement for SSRIs compared to TCAs. A study assessing the effects of the SSRI, fluoxetine, in hospitalized patients with depression showed a positive trend towards improvement of depression symptoms specially for those patients that were more severely ill (124).

In contrast to the wider general population or physically ill, at present, the data for efficacy of pharmacotherapy for anxiety and depression are limited for COPD. The main studies have been of SSRIs and TCAs. SSRIs are the first line pharmacotherapy treatment for depression and anxiety (125). For depression, small studies in COPD have shown mild improvements. Two studies of COPD patients using paroxetine showed variably significant improvement in quality of life questionnaires (126,127), and improvement of anxiety and depression scores and physical capacity after 3 months (127) (*Table 2*). A single-blinded open trial study of 57 COPD patients, aiming to assess the acceptability of fluoxetine therapy, showed that over two-thirds of patients declined to use fluoxetine therapy, mostly related to patient biases regarding use of psychiatric medication (128) (*Table 2*).

For TCAs, a number of small studies have been conducted in COPD. A study by Borson *et al.* showed that nortriptyline was effective in reducing depressive and anxiety symptoms and in increasing physical function (131), although a crossover study of similar size failed to show any benefit when using doxepin for patients with symptoms of anxiety and depression (129). In another study using protriptyline, the majority of the patients did not complete the trial because of the anticholinergic side effects (130). TCAs are no longer first line treatment for depression or anxiety, and consequently future trials for this medication class are unlikely (132).

Regarding the treatment of anxiety in COPD patients, a Cochrane review was unable to undertake any meta-analysis due to poor quality of the studies and very small sample sizes (122). Only four studies were analyzed, with two studies using SSRIs, and the other two using a TCA and azapirones. Two studies using SSRI showed a non-significant reduction in anxiety symptoms (122,127). The studies using TCA and azapirones did not show any improvement (129,133).

As was the case for psychological treatment, the overall effectiveness of pharmacotherapy for anxiety or depression in COPD has not been rigorously tested. Studies in COPD have been small, with large heterogeneity of sampling and tools used to assess efficacy of the treatments. In addition, there is limited evidence regarding the impact of side effects of pharmacotherapy, such as dry mouth and sexual dysfunction (123). Some side effects of treatment (such as dry mouth) may compound adverse effects of medications used for COPD, notably the anticholinergic activity of long-acting muscarinic antagonists (134). In addition, there are issues regarding patient refusal to take antidepressants due to misconceptions regarding depression and addiction, stigma associated with the disease, and lack of interest and motivation (132). Clearly, much more work needs to be done to test pharmacotherapy for anxiety and depressive symptoms in COPD, and to undertake head-to-head comparisons with psychological interventions and combinations of treatments (121).

The role of pulmonary rehabilitation

Pulmonary rehabilitation is an essential component of standard care for people who are symptomatic from chronic lung diseases causing breathlessness and functional impairment, such as COPD (135,136). Large observational studies of pulmonary rehabilitation participants have reported the prevalence of anxiety symptoms to range between 25% (137) and 32% (138), and depressive symptoms to range between 17% (137) and 27% (138). The symptoms of anxiety and depression have been associated with program non-completion (137,139), increased dyspnoea, fear of exercise and reduced functional performance both at commencement and completion of pulmonary rehabilitation (140,141). Furthermore, improvement in the symptoms of depression has been associated with improvements in specific domains of health-related quality of life (142). However, it is unclear if the symptoms of anxiety and depression should be addressed prior to entry to a pulmonary rehabilitation program or during the program.

Importantly, the symptoms of anxiety and depression have been shown to improve following completion of

Table 2 Example of studies involving pharmacotherapy and COPD for anxiety and/or depression

First author, year	Study design	Population	Measures	Results
Eiser et al. 2005 (127)	Double blinded RCT. Initial blinded for 6 weeks. After all patients took un-blinded Paroxetine for 3 months. Out-patients with moderate to severe, stable COPD. Patients screened using HAD and depression further diagnosed by psychiatrist	28 patient were diagnosed with depression. 14 patients in each group. 14 females and 14 males, mean age 66	HAD. BDI. Psychiatrist completed. MADRS. SGRQ. 6-minute walking test	6 weeks' treatment produced no significant differences between placebo. 5 patients treated with paroxetine developed side effects and medication was changed. Three months of un-blinded treatment: Significant improvement in HAD, BDI and MADRS. Significant improvement in walking distances (369 to 427 m, P=0.0003). Significant improvement in St. George's Respiratory Questionnaire Total Scores (65 to 58, P=0.033)
Lacasse et al. 2004 (126)	Double blinded randomized control trial. Treatment was paroxetine for 12 weeks. Outpatients with severe COPD, oxygen dependent. GDS >11/30	23 patients entered the trial. 82 refused. Treatment group (12 patients, 5 males, mean age 71.2. Control group (11 patients, 5 males, mean age 69.8	CRQ. SF-36. GDS. Side effects. Compliance	2 patients on paroxetine did not tolerate maximum dose. Clinical and statistical improvement in emotional domain of CRQ. Non significant improvement in GDS
Yohannes et al. 2001 (128)	Single-blinded (open) study. Paroxetine for 6 months. Inpatients with moderate to severe COPD. GMS diagnosed depression	57 (25 males and 32 females). Mean age 72. 14 accepted treatment with fluoxetine	BPQ, MRADL were done as baseline. MADRS	7 subjects completed the trial. 4 (57%) responded to fluoxetine therapy. 5 subjects withdrew because of side effects
Light et al. 1986 (129)	Double-blind crossover study. Doxepin or placebo for 14 weeks. Outpatients, at least moderately severe COPD	12 patients (all male), 6 patients for each group. 3 ceased doxepin due to side effects, 9 completed trial. Aged 57 to 69 (mean 61.2)	STAI. BDI. FEV_1, PaO_2, $PaCO_2$. 12 minute walk test	No improvement in any of the measures (anxiety and depression scores, respiratory function and physical capacity). Worse mean depression score for the treatment group. Half ceased therapy due to side effects (drowsiness, blurred vision, nausea and vomiting)
Ström et al. 1995 (130)	Double blind randomized trial. Outpatients, COPD stable with mild or moderate hypoxaemia. Treatment with Protriptyline for 12 weeks	26 patients. 14 patients on treatment group and 12 on placebo group. Treatment group had 4 males and 10 females. Aged 57 to 75 (mean 66). Placebo group had 4 males and 8 females. Aged 52 to 66 (mean 59)	SIP. MACL. HAD. Dyspnea score. Exacerbations. Arterial blood gas. Respiratory function	No improvement in all measures (arterial blood gas tension, spirometry, quality of life, anxiety and depression score, dyspnea score and exacerbations). 12 of 14 patients on protriptyline had side effects. 6 of 12 patients on placebo had side effects

HAD, Hospital Anxiety-Depression; BDI, Beck's Depression inventory; MADRS, Montgomery Asberg Depression Score; St. SGRQ, George's Respiratory Questionnaires; CRQ, Chronic respiratory questionnaire; GDS, Geriatric depression scale; GMS, The Geriatric Mental Status Schedule; MRADL, Manchester Respiratory Activities of Daily Living Questionnaire; BPQ, Breathing Problems Questionnaire; STAI, State-Trait Anxiety Inventory; MACL, Mood Adjective Check List; Hospital Anxiety and Depression (HAD) scale, Medical Outcomes Survey Short Form-36 (SF-36), Sickness Impact Profile (SIP) questionnaire.

comprehensive pulmonary rehabilitation (143,144). In a large randomised controlled trial, participants completing pulmonary rehabilitation were shown to significantly improve symptoms of anxiety and depression when compared to the control group of usual care (144). Studies have also shown that participants with symptoms of anxiety or depression can gain similar improvements in other program benefits arising from pulmonary rehabilitation. For instance, in an observational cross-sectional study, individuals with symptoms of anxiety and depression had similar benefits in exercise capacity and health-related quality of life following pulmonary rehabilitation as participants not experiencing these symptoms (145). Moreover, another observational study reported that participants with greater symptoms of anxiety in fact had a larger improvement from exercise training following pulmonary rehabilitation (146). Therefore, the recent guideline on pulmonary rehabilitation in adults from the British Thoracic Society states that the psychological status of participants is improved with pulmonary rehabilitation when compared with usual care, and recommends that individuals with symptoms of anxiety and depression should not be excluded from pulmonary rehabilitation (147).

Recommendations and future directions in research and practice

Full spectrum anxiety and depression are highly prevalent among patients with COPD and are associated with poorer outcomes. This seems to hold even for milder or sub-threshold levels of anxiety and depression. The first step to improve practice is to achieve earlier and more accurate diagnosis of these psychological comorbidities in COPD. This is important since these conditions are underdiagnosed and consequently undertreated (8,58). Self-reported screening instruments are useful as an initial approach; however validated tools should then be utilized to minimize false positives and standardize care. When and in whom screening should be done is still not clear for patients with COPD. It is also not clear if it should be carried out with all COPD patients or just to those at higher risk of these comorbidities. After the psychological distress screening scale has been performed, high-scoring patients should be referred to a mental health specialist to facilitate access to comprehensive, gold-standard diagnostic assessment (85).

Due to the impact of associated depressive and anxiety disorders and symptoms on COPD patients, determining the best treatment approach is essential. Unfortunately, as

highlighted in this review and by others, there is currently a relative scarcity of strong evidence of benefit for any specific pharmacological or non-pharmacological treatment for anxiety and depression in COPD (4). Furthermore, at this point of time, guidelines are based on treatment of depression and anxiety for the general population (3).

Due to the bidirectional nature of the association of COPD with depression and anxiety, an integrated approach that enhances the benefits between mental and physical health would be the most effective. There is extensive evidence of the benefits of pulmonary rehabilitation for patients with COPD and it has shown to significantly reduce symptoms of both anxiety and depression in COPD patients, possibly through improved physical capacity (148). Adding a depression or anxiety targeted treatment to the pulmonary rehabilitation program may have additive therapeutic benefits. This synergistic effect has been alluded to in a study where marked improvement in depression symptoms was shown when brief inpatient pulmonary rehabilitation plus antidepressants were used with COPD patients with major depression (149). Similarly, another study showed a significant improvement in anxiety and depression with improvement of physical capacity, when CBT was provided within a pulmonary rehabilitation program (101).

Future studies should aim to fill the current gaps in knowledge about treatment of psychological symptoms in COPD. First there are no large studies that have definitively assessed the true benefits of psychological, pharmacological or combined treatment modalities in the COPD population. Future studies should also focus on determining the best treatment for specific COPD groups e.g., based on gender, severity of COPD and frequency of exacerbations. There is also uncertainty regarding the cost-effectiveness of targeted treatment of anxiety and depression, and feasibility of restructuring health-care delivery to incorporate care for mood and anxiety disorders as an integral part of high quality, comprehensive chronic disease management of patients with COPD.

Summary

This review has provided an overview of the pathophysiology, prevalence and impact of anxiety and depression in patients with COPD, and has discussed diagnosis and treatment options for these important psychological comorbidities. In COPD patients, the presence of symptoms of anxiety and depression are common and have significant impacts that, adversely affect mortality rate, exacerbation rates,

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hospital length of stay, quality of life and functional status. Anxiety and depression are underdiagnosed in patients with COPD, and consequently undertreated. Studies examining specific pharmacological and non-pharmacological treatment of these conditions are limited and generally are comprised of small studies of varying quality. Given the current state of knowledge, many further areas of research are needed in the field of COPD chronic disease management, including in whom to screen for clinically important anxiety and depression, and the most effective and cost-effective treatment approaches for these conditions in COPD patients. A much greater awareness of the clinical importance of mental health comorbidities in COPD is urgently needed.

Acknowledgements

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Biomarkers of progression of chronic obstructive pulmonary disease (COPD)

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Abstract: Disease progression of chronic obstructive pulmonary disease (COPD) is variable, with some patients having a relatively stable course, while others suffer relentless progression leading to severe breathlessness, frequent acute exacerbations of COPD (AECOPD), respiratory failure and death. Radiological markers such as CT emphysema index, bronchiectasis and coronary artery calcification (CAC) have been linked with increased mortality in COPD patients. Molecular changes in lung tissue reflect alterations in lung pathology that occur with disease progression; however, lung tissue is not routinely accessible. Cell counts (including neutrophils) and mediators in induced sputum have been associated with lung function and risk of exacerbations. Examples of peripheral blood biological markers (biomarkers) include those associated with lung function (reduced CC-16), emphysema severity (increased adiponectin, reduced sRAGE), exacerbations and mortality [increased CRP, fibrinogen, leukocyte count, IL-6, IL-8, and tumor necrosis factor α (TNF-α)] including increased YKL-40 with mortality. Emerging approaches to discovering markers of gene-environment interaction include exhaled breath analysis [volatile organic compounds (VOCs), exhaled breath condensate], cellular and systemic responses to exposure to air pollution, alterations in the lung microbiome, and biomarkers of lung ageing such as telomere length shortening and reduced levels of sirtuins. Overcoming methodological challenges in sampling and quality control will enable more robust yet easily accessible biomarkers to be developed and qualified, in order to optimise personalised medicine in patients with COPD.

Keywords: Pulmonary disease; chronic obstructive; disease progression; biological markers (biomarkers); lung; sputum; blood

Chronic obstructive pulmonary disease (COPD) is a chronic, inflammatory lung disease that arises from exposure to cigarette smoke and other inhaled toxins, and results from a gene-environment interaction (1). Disease progression of COPD is variable, with some patients having a relatively stable course, while others suffer relentless progression leading to severe breathlessness, frequent acute exacerbations of COPD (AECOPD), respiratory failure and death. This review will initially focus on radiological markers, and biological markers (biomarkers) in lung tissue, sputum and blood, which may be useful in predicting disease progression in COPD. Emerging approaches to discovering markers of gene-environment interaction will then be discussed, including exhaled breath analysis, exposure to air pollution, the lung microbiome, and lung ageing.

Measurements of disease progression in patients with COPD

Decline in lung function has been the classical objective

measure of progression of COPD over time. However, other clinically important measures have been used in epidemiological studies and clinical trials, including symptoms and health status, exacerbations and health care utilisation, and mortality.

Lung function

Lung function, particularly the forced expiratory volume in 1 second (FEV_1), provides an objective, physiological measure of worsening airflow obstruction in COPD. The classic Fletcher and Peto study (2) described variable decline in lung function in a cohort of male workers, with some smokers being more susceptible to accelerated decline. A range of clinical and demographic factors has been shown to influence decline in lung function in COPD, such as environmental and occupational pollutants, cigarette smoking, respiratory infections, exacerbations and comorbidities (3). The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort study of 2,163 patients observed a mean rate of decline in FEV_1 of 33 mL/year, with higher rates of FEV_1 decline in the presence of current smoking, emphysema and bronchodilator reversibility (4).

Symptoms and exacerbations

The Global Initiative for chronic obstructive lung disease (GOLD) guidelines recommend the inclusion of symptom assessment and exacerbation history, together with measurement of the severity of airflow limitation measured by FEV_1 (5). The ECLIPSE study confirmed that patients with 2 or more exacerbations per year (frequent exacerbators) were at higher risk of future exacerbations, and this risk was further increased with more severe airflow limitation (6). Change in health status is also an important measure of disease progression (1).

Mortality

COPD is the third leading cause of death world-wide, after ischaemic heart disease and stroke (7). This high rate of mortality is driven by increased smoking worldwide, reduced mortality from other treatable diseases and an ageing world population (5). Groups at high risk of mortality, as described in the Copenhagen City Heart Study of 10,457 participants, included those with lower baseline FEV_1 and excessive longitudinal decline in FEV_1,

even before the point where their lung function becomes abnormal (8).

It is evident that a multitude of relevant clinical phenotypes portrays the disease progression of COPD, which reflects the heterogeneous and complex nature of this chronic disease.

Biomarkers for disease progression of COPD

Biomarkers are any clinical features, imaging quantification or laboratory-based test markers that characterise disease activity, which are useful for diagnosing and monitoring disease processes and response to therapy. Recent excellent reviews have summarised putative biomarkers for detecting the presence of COPD, characterising COPD phenotypes and monitoring response to treatment (9-11). Biomarkers of acute exacerbations have also been reviewed (12).

Identifying individuals with COPD who are at higher risk of progression would enable more personalised management, in order to slow disease progression. Use of biomarkers would potentially add to existing strategies for smoking avoidance, pharmacotherapy, pulmonary rehabilitation and chronic disease management in COPD. Benefits from measuring biomarkers for COPD progression (and not only susceptibility to COPD) include identifying patients who are rapid decliners in the early stages of the disease, predicting disease progression in all severity groups of COPD, and quantifying response to treatment.

The search for reliable biomarkers in COPD, other than FEV_1, is ongoing [e.g., the international efforts by the COPD Biomarker Qualification Consortium (9)]. Providing reliable evidence to validate biomarkers before clinical implementation remains an important challenge. Important issues to be addressed include the accuracy and reliability of biomarkers for the clinical state of interest, evaluation of clinical utility and cost-effectiveness, and real world effectiveness compared to other biomarkers (13). The validation of biomarkers (biomarker qualification) for COPD would be clinically applicable to risk stratification of patients and outcome markers of efficacy and safety in drug development and other clinical trials (9).

Radiological markers for emphysema, airway thickness, bronchiectasis and multi-morbidities

Image biomarkers, especially radiological features of COPD morphology visualised on high resolution computed tomography (CT) chest scans, have been found to be useful

predictors of disease progression.

Emphysema and airway wall thickness

High resolution CT is able to assess emphysema and airway disease using quantitative indices (14). Inspiratory *vs.* expiratory analysis of distribution of parenchymal (emphysema) and functional small airways disease provides information about COPD phenotype (15), and change in lung density over time can itself be measured as an endpoint of COPD progression (16).

Quantitative CT measurements have been associated with outcomes of COPD progression in large cohort studies. Accelerated decline in lung function has been associated with more severe emphysema measured quantitatively by CT (17). The MESA (Multi-Ethnic Study of Atherosclerosis) study found that the presence of centrilobular and panlobular emphysema correlated with increased dyspnoea and reduced exercise capacity (18). Airway wall thickness correlated with reduced lung function and increased symptoms in smokers in a cross-sectional study (19). In the COPDGene study of 1,002 subjects, exacerbations were more frequent in those subjects who had a more severe CT emphysema index, and who displayed increased airway wall thickness (20). A higher CT emphysema index was associated with increased risk of respiratory (21,22) and COPD-specific mortality (23). Airway wall thickness was not independently associated with mortality (22).

Bronchiectasis

Bronchiectasis frequently coexists with COPD. Bronchiectasis is a persistent or progressive condition that is characterised by dilated, thick-walled bronchi that fail to clear airway secretions normally. This leads to bacterial infection and a chronic cough productive of sputum, recurrent infective exacerbations and ultimately, lung destruction and respiratory failure (24). In some COPD patients, bronchiectasis is an incidental finding on CT and may be subclinical, as observed in the ECLIPSE study where the overall prevalence of bronchiectasis was 4% in a highly selected population of milder COPD patients (25). In contrast, studies of moderate to severe COPD have demonstrated a higher prevalence of bronchiectasis of from 30% to 60%, with more extensive bronchiectasis in severe COPD (26-28).

The presence of bronchiectasis influences respiratory infections and other complications of COPD. In a study of patients with moderate to severe COPD, patients with COPD and co-existing bronchiectasis, compared to COPD alone, had more severe airflow obstruction (OR 3.9) and an increased yield of potentially pathogenic microorganisms on sputum culture (OR 3.6) (29). Furthermore, bronchiectasis increased the rate of at least one hospital admission for an AECOPD in the previous year (OR 3.0). In a subsequent study of 201 patients with moderate to severe COPD, the same investigators showed that bronchiectasis was independently associated with increased all-cause mortality (HR 2.5) (30). Conversely, a study of 245 patients with non-cystic fibrosis bronchiectasis in Belgium found that 17% of patients had co-existing COPD (31). Over 5 years of follow-up, patients with both bronchiectasis and COPD had a mortality rate of 55%, which was considerably higher than 13% in patients with bronchiectasis alone (31).

These studies emphasise the clinical impact of coexisting bronchiectasis in patients with COPD, especially in terms of excessive rates of AECOPD and mortality. Detecting bronchiectasis in patients with COPD from their routine HRCT chest scans may therefore be potentially clinically useful, identifying those patients who are predisposed to higher rates of exacerbations and increased mortality.

Coronary artery calcification (CAC)

Cardiovascular multi-morbidity is highly prevalent in patients with COPD, and adversely affects mortality. A high prevalence of coronary artery disease has been associated with emphysema severity (32). CAC is a marker of coronary artery disease (*Figure 1*), and its extent is directly associated with the total burden of coronary atherosclerosis (33). Whilst CAC can be measured using calcium scores on gated, non-contrast CT scans, the use of simple visual scores of CAC has also found utility in lung cancer screening studies (34). In a cross-sectional study of 200 patients with moderate to severe COPD, we observed a high prevalence of CAC (87%) on routine CT chest scans (35). Of prognostic importance, a moderate to high ordinal visual score for CAC (>4 out of a possible 12) was predictive of increased all-cause mortality (HR 2.0) in these patients with COPD (35) (*Figure 2*). This association was independent of duration of cigarette smoking. Similarly in the ECLIPSE study, a higher coronary artery calcium score percentile was associated with increased mortality (HR 1.77) in COPD patients (36). The results of these radiological studies suggest the scoring of CAC severity on CT chest

Severe CAC on mediastinal windows: *Severe emphysema on lung windows*:
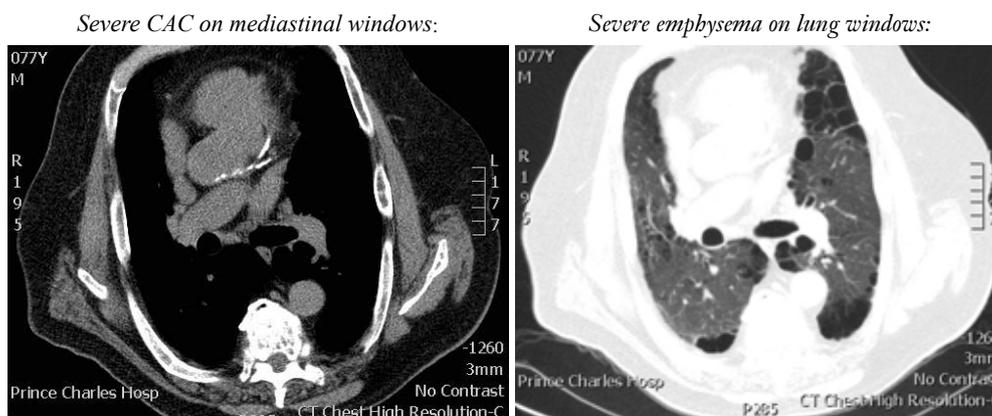
Figure 1 CAC on CT chest scan in a 77-year-old male patient with COPD and emphysema. CAC, coronary artery calcification; CT, computed tomography. Reproduced with permission from (3).
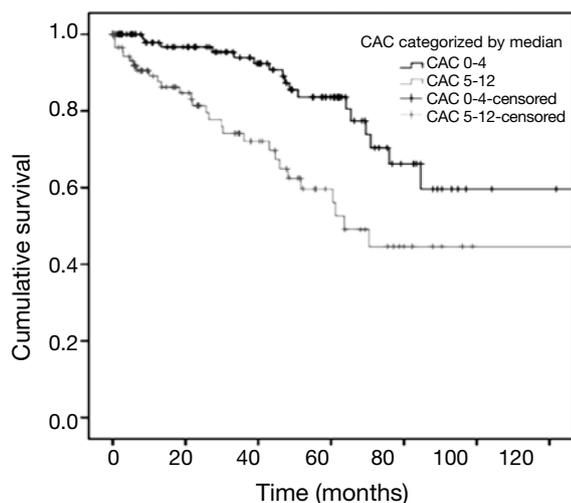
Figure 2 Kaplan-Meier survival curves for all-cause mortality for COPD patients, according to CAC score >4 (CAC 0-4 n=109, CAC 5-12 n=91). The Kaplan-Meier survival curve (censored at 120 months) shows that COPD patients with CAC scores of 5 to 12 (dichotomised by the median of 4) had higher mortality than patients with CAC scores of 0 to 4 (log rank test, P=0.001). CAC, coronary artery calcification. Reproduced with permission from (35).

scans can non-invasively screen for coronary artery disease in patients with COPD with important prognostic implications.

Lung tissue: gene expression markers

Molecular changes in lung parenchyma are a direct reflection of alterations in lung pathology that occur with disease progression in COPD. Routine collection of lung samples is only feasible in patients undergoing lung surgery. Nevertheless, molecular changes in lung tissue provide valuable insight into biomarkers that may be expressed and therefore usefully measured in accessible samples (e.g., sputum, exhaled breath condensate and blood).

A number of studies have used microarrays to examine differences in global mRNA expression between chronic lung disease and normal lung samples (37,38). Other studies have extended this approach by profiling gene expression across different severity stages of COPD. A study of lung tissue from COPD patients (n=21 GOLD stage 0; n=9 stage I; n=10 stage II; n=3 stage III) showed that gene expression correlated with forced expiratory flow between 25% and 75% of forced expiratory volume ($FEF_{25-75\%}$), a measure of small airways function (39). Upregulated genes included those involved in pathways of apoptosis and extracellular matrix synthesis and degradation; down-regulated genes included anti-inflammatory genes. A study of 56 lung tissues (no COPD, to COPD patients from mild to severe) found correlation of FEV_1 % predicted and FEV_1/FVC with functional classes of genes involved in DNA binding and transcription (40).

Studies from our group have also provided evidence for differences in gene expression signatures in the earlier stages of COPD disease progression. We have undertaken a study of lung tissue of 30 smokers with emphysema undergoing lung resection for lung cancer, with biological validation in an independent set of 62 patients (41). All patients had airflow limitation with FEV_1/VC ratio <0.70 and were arbitrarily classed, based on gas transfer, as mild (KCO ≥75% of predicted) or moderate (KCO <75% of predicted) emphysema. Gene expression profiling
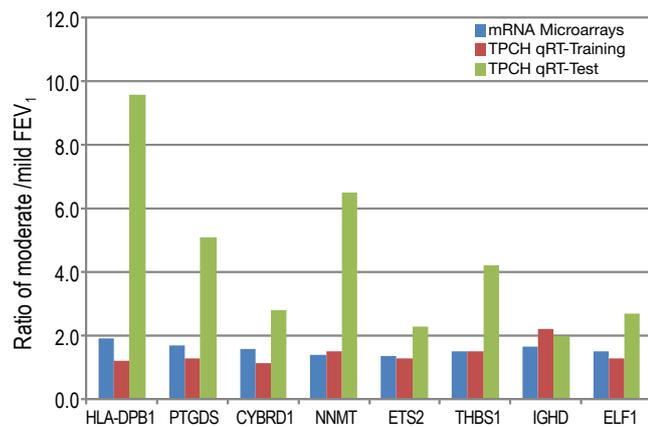
Figure 3 Comparison of mRNA expression differences in non-tumour lung tissue between mild and moderate COPD patients, based on FEV$_1$ % predicted. Fold-changes are shown for eight candidate genes measured by gene expression microarrays (TPCH training set, n=30), and qRT-PCR (technical replication: TPCH training set, n=30; biological validation: TPCH test set, n=58). NNMT, nicotinamide N-methyltransferase; THBS1, thrombospondin 1; IGHD, immunoglobulin heavy delta chain; HLA-DPB1, major histocompatability complex, class II, DP Beta 1; PTGDS, prostaglandin D2 synthase; CYBRD1, cytochrome B-Reductase 1; ETS2, erythroblastosis virus E26 oncogene homologue 2; ELF1, E74 like factor 1. Reproduced with permission from (42).

and confirmatory PCR identified seven genes that were differentially expressed in moderate emphysema, compared to mild emphysema by more than 1.3-fold: *COL6A3*, *SERPINF1*, *ZNHIT6*, *NEDD4*, *CDKN2A*, *NRN1* and *GSTM3* (41). Our additional study of lung tissue from patients with mild (n=9) or moderate (n=9) COPD, based on FEV$_1$ % predicted, with validation in an independent set of 58 lung samples, confirmed differential expression of eight genes (*NNMT*, *THBS1*, *HLA-DPB1*, *IGHD*, *ETS2*, *ELF1*, *PTGDS* and *CYRBD1*) by more than 1.8-fold between mild and moderate COPD severity (42) (*Figure 3*). Ontologies represented by these genes were predominantly cell migration, proliferation, angiogenesis and apoptosis (42). Using the same lung tissue, we have also shown that expression of microRNA-34c is associated with emphysema severity, and modulates SERPINE1 expression in COPD lung (43). Genes and pathways associated with severity of COPD, including the early stages of progression, could therefore be tested as lung biomarkers for progression of emphysema and airflow obstruction in COPD.

Gene expression profiling of specific cells or regions of COPD lung provide additional information about distinct gene signatures for disease progression. Expression of repair genes was examined in 136 paired small airways and emphysema lung tissue obtained by laser capture microdissection from 63 patients (44). Genes involved in tissue destruction were more commonly increased in expression in emphysematous lung tissue and correlated with impaired FEV$_1$, whereas these genes were not as highly expressed in the small airways, thereby promoting bronchiolar remodelling rather than destruction (44). In a study of 238 smokers with or without COPD, gene expression in bronchial brushings was similar to the expression in lung tissue, and this gene expression was regulated in part by activating transcription factor 4 (ATF4) (45). Finally, distinct gene signatures were observed in fibrotic and emphysematous areas of lung, in patients with combined pulmonary fibrosis and emphysema (46). Fibrotic regions expressed genes associated with immune function, and emphysematous areas expressed genes related to cellular fraction, membrane biology, and vascular biology (46), demonstrating that functional differences in gene expression occur with different lung pathologies. Overall, these studies show that specific cells and pathologies in the lung are likely to yield characteristic biomarkers that reflect individual COPD phenotypes of progression.

Sputum: inflammatory cells and mediators

Sputum has been studied as a non-invasive method of sampling biomarkers to assess disease severity and progression in COPD, including exacerbations. Many COPD patients can produce spontaneous sputum samples. However, these often contain a high proportion of non-viable cells which may influence the cell count and mediator profile. To overcome this, sputum can be induced with hypertonic saline in stable patients with COPD, with good safety and reproducibility for cell counts and inflammatory markers (47). Induced sputum also has an adequate safety profile during acute exacerbations, as demonstrated in studies of patients with mild to moderate (48) and moderate to severe COPD (49). Because of many technical and clinical confounding factors (such as interference with assays, smoking status of patients, bacterial infection and concomitant treatment), induced sputum is still undergoing investigation as a source of clinically useful biomarkers (9).

Sputum biomarkers during stability have been associated with severity of COPD. Sputum neutrophil count increased

with GOLD stage but was only weakly associated with lung function in the ECLIPSE study (50). Higher levels of human neutrophil peptides (HNP), neutrophil elastase (NE), interleukin (IL)-8 and matrix metalloproteinase (MMP)-9 in spontaneous sputum of COPD patients were associated with greater decline in lung function (FEV_1) over 2 years (51). In the ECLIPSE study, microarray profiling of gene expression in induced sputum from 148 COPD patients (and validated in 176 patients) found 277 genes differentially expressed between moderate, severe and very severe GOLD classes, and 198 genes that were differentially expressed between severities of emphysema (52). Further validation is required to test the clinical utility of these genes as biomarkers for COPD progression.

During exacerbations, sputum cell and mediator profiles are heterogeneous and can predict response to therapy of the exacerbation (53). The presence of a mixed inflammatory cell profile in the sputum, together with increased concentrations of sputum and serum biomarkers, were found in patients with exacerbations who had lower FEV_1 and increased hospital length of stay (53). Inflammatory mediators in induced sputum during stability may predict future risk of exacerbations. A review by Koutsokera and co-workers found that levels of some mediators in sputum [including in sputum IL-6, IL-8 and myeloperoxidase (MPO)] may be associated with frequency of exacerbations, although more confirmatory studies are needed (12). In a longitudinal study with monthly visits, sputum levels of leukotriene B_4 were found to be elevated prior to an exacerbation, and were suggested as possible biomarkers for exacerbation risk (54).

Blood biomarkers: monitoring the systemic compartment

Blood samples provide a convenient source of biomarkers of lung disease. The relevance of blood biomarkers depends on release of markers from the lung into the bloodstream, or systemic markers present in the blood that reflect active disease processes in the lung.

A range of blood biomarkers has been associated with severity of airflow limitation and emphysema. Reduced levels of serum club (Clara) cell protein 16 (CC-16), a protein produced in the lungs and released to the serum, were weakly associated with accelerated decline in lung function (FEV_1) in both the Lung Health Study (55) and ECLIPSE study (4). In the TESRA (Treatment of Emphysema with a Selective Retinoid Agonist) and ECLIPSE studies, reduced serum

levels of soluble receptor for advanced glycation endproducts (sRAGE) were associated with more severe GOLD stage and more extensive emphysema (56). Lower levels of sRAGE were similarly associated with more advanced emphysema or lower FEV_1 in two other studies (57,58). In the ECLIPSE cohort, low levels of vitamin D were correlated with FEV_1 and severity of emphysema and associated with 6-minute walk distance, bronchodilator response and CC-16 levels (59). In a subset of the COPDGene cohort, emphysema quantified on CT was associated with higher plasma levels of the adipokine, adiponectin (60) and lower levels of plasma IL-16 (61). Plasma YKL-40 has been associated with higher all-cause mortality (HR 1.4) in a cohort of 493 COPD patients in Denmark (62). Thus a range of biomarkers detectable in peripheral blood show potentially promising relationships with COPD phenotypes.

Panels of blood biomarkers may provide more accurate modelling of future risk. In the Grosshansdorf COPD cohort of 140 COPD patients, clusters of plasma proteins involved in neutrophil function were associated with parameters related to FEV_1 (63). Furthermore, proteins related to the epidermal growth factor receptor (EGFR) pathway were associated with gas transfer (DLCO) and FEV_1 (63). A panel of three systemic inflammatory markers in peripheral blood (CRP, fibrinogen and leukocyte count) was tested in 6,574 individuals with COPD (defined as FEV_1/VC ratio <0.7) in the Copenhagen City Heart Study and the Copenhagen General Population Study (64). Elevation of all three biomarkers simultaneously was associated with an increased risk (OR 3.7) of having frequent exacerbations. This association was observed even in subjects with milder COPD and those with no history of frequent exacerbations (64). In the ECLIPSE study, adding the full range of studied blood biomarkers [white blood cell counts, fibrinogen, chemokine ligand 18, surfactant protein D, CRP, Clara cell secretory protein-16, IL-6, IL-8, tumor necrosis factor α (TNF-α)] to the model of age, BODE index and previous COPD hospitalisations improved prediction of mortality (65). A refined panel of six systemic inflammatory markers in peripheral blood (white cell count, fibrinogen, CRP, IL-6, IL-8, TNF-α) in the ECLIPSE study was able to predict increased mortality and exacerbation rates in COPD patients with inflammation, compared to patients without inflammation (66). Of these, currently plasma fibrinogen is being considered for regulatory qualification as a prognostic marker by the US Food and Drug Administration and the European Medicines Agency (9,67).

Because of the large number of putative biomarkers, heterogeneity in study design and evaluation, a formal systematic review of this emerging field is beyond the scope of this review.

Emerging gene-environment approaches to biomarkers of disease progression in COPD

In addition to the more traditional sampling of biomarkers described above, emerging approaches to capturing the effects of gene-environment interaction on COPD disease progression are receiving more focus in research studies. Of these, analysing biomarkers in exhaled breath is a potentially useful, non-invasive method of sampling the airways and epithelial lining fluid that is exposed to the environment. In addition to cigarette smoking, exposure to air pollution and infection are important environmental drivers of COPD progression and phenotypes. Finally, lung ageing, whilst an endogenous chronological factor, also brings with it exposure to internal and external factors over many years, and should be integrated into the complex profiling of COPD.

Exhaled breath analysis

Volatile organic compounds (VOCs)

Advances in technology have produced small, portable array type devices (electronic noses) that are highly applicable to the clinical setting. Electronic noses use a variety of technologies to emulate the human nose, with VOCs adsorbing onto sensors to produce a change in conductivity, colour or oscillation of a crystal, leading to readouts that are analysed. These devices approach the problem of detection from an entirely different viewpoint from that of the gas chromatograph: in the same way a human nose can tell the difference between the bouquet of chocolate and a rose without needing to know the chemical constituents of the vapour, so the electronic nose is able to discriminate between two vapour mixtures without needing to characterise the exact molecules responsible.

Exhaled breath analysis using differing technologies, including gas chromatography-mass spectrometry and the electronic nose, can discriminate between a range of pulmonary diseases (68), including COPD and asthma (69,70). Relatively few studies to date have linked VOCs profiling of the exhaled breath with COPD progression. A recent study showed that the VOCs pattern is reasonably reproducible in healthy subjects and patients with severe COPD and has some correlation with tests of small airways

disease (71). VOCs pattern was shown to differentiate between some phenotypes of COPD, such as patients with higher sputum eosinophilia or frequent exacerbations (72).

Identifying the neutrophilic and eosinophilic inflammatory phenotypes of COPD would further aid in tailoring effective treatment. A strong association between sputum cell count and exhaled breath compounds has been demonstrated in subjects with mild to moderate COPD (GOLD stages I and II) (73). Moreover, VOCs profiling was able to discriminate between subjects with COPD and α_1-antitrypsin (AAT) deficiency, with very high accuracy, and the VOCs profile of AAT deficiency patients changed with human recombinant AAT therapy, indicating a possible marker of response to treatment (74). However, before widespread application in the clinical setting, methodological issues of VOCs testing need to be overcome, and more extensive validation is required (75).

Exhaled breath condensate (EBC)

Collection of cooled exhaled breath as condensate is a non-invasive method of sampling the airway lining fluid (76). To date, a small number of studies have examined EBC biomarkers and COPD progression. EBC pH was found to be lower in former smokers with GOLD stage III to IV COPD, compared to stage I (77), suggesting that airway acidification could be a marker of airway inflammation and disease severity in COPD, although not all studies have shown a relationship with FEV_1 (78). EBC pH is also reduced during acute exacerbations (79). EBC hydrogen peroxide (H_2O_2), a marker of oxidative stress, has been shown to correlate with COPD health status as measured by the COPD assessment test (CAT) (80). Methodological issues such as dilution and sensitivity of assays, as well as interpretation of clinical factors that impact on EBC analysis, still require to be solved in larger studies (9).

Exposure to air pollution

The predominant sources of particulate matter in the lungs of COPD patients are cigarette smoke and ambient air pollution (81). With up to 25-45% of patients with chronic airflow limitation being never smokers (82), it is evident that non-smoking-related factors (e.g., air pollution) play a role in the progression of COPD (82,83). Exposure to air pollution should therefore be characterised as a factor that influences disease outcomes in COPD.

Vehicle emissions are a major contributor to air pollution

in the urban environment. The main components of vehicle emissions are particulate matter less than 10 μm in diameter (PM_{10}), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) (84). Recent epidemiological studies have observed strong associations between air pollution exposure and COPD outcomes, including exacerbations, hospital admissions and mortality (*Table 1*). The repetitive nature of the inhalation injury caused by air pollution is considered a major mediator in the COPD progression (81). Chronic exposure to air pollution, specifically vehicle emissions, has been linked to increased hospital admissions of COPD patients, including those who are never smokers (89). Analysis of early evidence showed that long-term exposure to particulate matter can lead to a reduction in lung function and increased COPD incidence and progression (81). These studies and others (86,87) support the notion that exposure to air pollution is a driver of COPD progression in susceptible individuals.

Monitoring of air quality occurs for legislative and public health requirements, as well as epidemiological research. However, real-time monitoring of personal air pollution exposure and biomarkers of the adverse effects of ambient air pollution are still in development (93). *In vitro* studies have elucidated gene and protein expression profiles of human bronchial epithelial cells, in response to air pollutant exposure (3), which could be brought to clinical testing with further validation. EBC levels of nitrite and nitrate (markers of oxidative stress) were associated with concentrations of ambient coarse particles, but not indoor air pollutant levels, in four cities in Europe (94). Systemic responses to air pollutants were studied in 242 stable COPD patients in Spain (95). In this time series analysis, blood levels of CRP, fibrinogen, HGF and IL-8 were associated with increased ambient NO_2 levels, mainly detected in former smokers.

At present, little is known about the molecular mechanisms by which air pollution can promote progression of COPD, and further studies are needed in this field.

Lung microbiome

Bacteria are strongly associated with AECOPD, with bacteria cultured in ~50% of patients with an AECOPD (96). Chronic airway infection with bacteria (colonisation of the airways by bacteria) is more common in patients with severe COPD (97). Whether chronic infection contributes to the pathogenesis of airway inflammation and increasing frequency and severity of AECOPD is not known. The 'vicious circle' hypothesis outlines the principles that chronic microbial colonisation, alters innate immunity and airway epithelial injury contributes to the progression of both COPD and other chronic lung diseases such as bronchiectasis (28,98). According to this paradigm, the presence of chronic bacterial infection in the airways, (including during stable disease), may drive inflammation and disease outcomes.

The microbiome describes the microbial community that share an environment in a particular body site. Next-generation sequencing is used to identify these microbial populations which include microbes that are unculturable (99). Characterising the microbiome is rapidly emerging as an important approach to unravelling the complex microbiology of chronic lung diseases (100) [outlined in detail in this issue of the Journal by Daniel Chambers and colleagues (101)]. The community composition of microbial communities can be determined by sequencing the variable regions of the 16S gene, which encodes bacterial ribosomal RNA (rRNA) (98). Published studies of the lung microbiome in COPD have recruited relatively small numbers of patients, with a range of methods of sampling the microbiome (*Table 2*). Furthermore, few studies to date have applied study of the lung microbiome to outcomes of COPD progression. In general, tobacco smoking in the absence of COPD does not appear to alter the lung microbiome, but severe COPD is associated with less population diversity of resident bacterial communities, although even this result seems dependent on whether BAL or airway tissue is being sampled (96,103,106).

In COPD, bacterial community profiles in BAL samples from patients using inhaled steroids and long-acting bronchodilators clustered differently from the profiles observed in patients not using these medications (105). Infection with respiratory viruses increases the total bacterial load in patients with COPD, compared to similarly infect healthy controls, but with no obvious difference in bacterial diversity based on analysis of sputum samples (106).

These alterations to the lung microbiome have considerable potential implications for the pathogenesis and progression of COPD. Predominance of one bacterial species in an anatomical lung region (e.g., affected by bronchiectasis) could reduce bacterial diversity, leading to disruption of the balance between mucosal immunity and the bacterial communities present (airway dysbiosis). Alteration of the normal balance of bacterial flora may lead to an excessive inflammatory response, perpetuating the airway inflammation that is characteristic of COPD (98). The microbiome is an emerging source of biomarkers of respiratory infection and possibly COPD progression.

Table 1 Examples of recent studies of air pollution exposure and COPD outcomes

Study	Location	Study design	Main findings
Waked 2012 (85)	Lebanon	• Cross-sectional analysis of Lebanese residents over 40 years from October 2009 to September 2010 • Out of 2201 individuals, 732 were never smokers, and 3.4% had COPD • Lung function and exhaled carbon monoxide levels were measured	• Correlation between COPD incidence and indoor air pollution from house warming with diesel, as well as childhood respiratory disease and older age
Gan 2013 (86)	Canada	• Longitudinal study with a 5-year exposure period and 4 year follow up period • 467,994 participants, with no COPD baseline and an age range between 45-85 years old	• Black carbon was strongly associated with risk of COPD hospitalization and mortality (which was attenuated when demographic factors such as age and sex were accounted for) • 6% increased risk of mortality and 7% increased risk of hospitalization with higher levels of exposure
Hansel 2013 (87)	USA	• Longitudinal study to investigate the effects of indoor PM and NO_2 concentrations on COPD morbidity in a peri-urban community • Pollutant concentrations measured at baseline, 3 and 6 months • 84 participants with moderate to severe COPD	• Indoor pollutant exposure to $PM_{2.5}$ and NO_2 was associated with increased respiratory symptoms and risk of COPD exacerbations
Wang 2013 (88)	Taiwan	• Time series analysis of outpatient visits and air pollution in the context of a heavily polluted urban area	• NO and NO_2 were positively associated with respiratory disease, but also some association was seen for PM_{10}, $PM_{2.5}$, O_3, CO and SO_2 • COPD outpatients were most sensitive to air pollution and weather
Schikowski 2014 (89)	Europe	• Impact of chronic exposure to air pollution on COPD in four cohorts • The annual average of PM_{10}, NO_2 and road traffic exposure was assessed	• In meta analyses, NO_2, SO_2, PM_{10} and the traffic indicators were positively associated with COPD • Significant positive association with traffic intensity and COPD incidence in never smokers
Tao 2014 (90)	China	• Time series analysis of PM_{10}, SO_2 and NO_2 and respiratory hospitalizations • 28,057 recorded admissions for respiratory disease	• Significant positive association between air pollutants and respiratory hospitalizations, and stronger effects were observed for females and persons aged ≥65 years
Zhou 2014 (91)	China	• Cohort of 71,431 middle-aged Chinese men from 25 different cities • Annual average particulate matter exposure was estimated and compared to total, cardiovascular and respiratory disease mortality	• 1.7% increase in respiratory mortality per 10 µg/m^3 increase in PM_{10}
Vanos 2014 (92)	Canada	• Investigation into the relative risk of mortality from all non-accidental, respiratory, and cardiovascular related causes, associated with exposure to four air pollutants, by weather type and season, in ten major Canadian cities	• In total, 61% of the respiratory-related mortality relative risk estimates were significantly higher than for cardiovascular-related mortality. The combined effect of weather and air pollution is greatest when tropical-type weather is present in the spring or summer

COPD, chronic obstructive pulmonary disease; PM_{10}, particulate matter less than 10 µm in diameter; NO_2, nitrogen dioxide; SO_2, sulfur dioxide.

Table 2 Microbiome studies of COPD patients and smokers

Study	Population	Main findings
Hilty 2010 (102)	11 asthma, 5 COPD, 8 controls—bronchial brushings	Microbiota present in bronchial tree. Proteobacteria more frequent in COPD and asthma
Huang 2010 (96)	8 COPD, mechanically ventilated—endotracheal aspirates	Presence of diverse bacterial communities in airways of COPD patients with severe exacerbation
Erb-Downward 2011 (103)	4 COPD, 7 smokers, 3 never-smokers—BAL; 6 severe COPD—lung tissue explants	Lung microbiome distinct from oral microbiome. Lower diversity in more severe COPD; Pseudomonas predominance. Differences in bacterial communities within lung sites in severe COPD
Cabrera-Rubio 2012 (104)	6 moderate COPD—sputum, bronchial wash, bronchial biopsy, BAL	High bacterial diversity. Sputum and washings – different microbiota to BAL and biopsies
Pragman 2012 (105)	22 moderate to severe COPD, 22 controls—BAL	Increase in microbial diversity in COPD. No difference with increasing severity. Differences based on inhaler therapy
Sze 2012 (99)	8 severe COPD, 8 CF, 8 smokers, 8 never-smokers—lung tissue	Lower bacterial density and differences in bacterial populations in severe COPD lung tissue
Molyneaux 2013 (106)	14 COPD, 17 controls infected with rhinovirus—induced sputum	Increase in bacterial burden in COPD after RV infection, including Haemophilus influenzae
Morris 2013 (107)	19 smokers, 45 non-smokers—BAL, oral wash	Higher abundance of some bacteria in the lungs. No difference between smoker and non-smoker lung microbiota
Zakharkina 2013 (108)	9 COPD, 9 controls—BAL	Diverse bacteria present in healthy lungs and COPD, different bacterial taxa in COPD
Galiana 2014 (109)	9 mild/moderate COPD, 10 severe COPD—sputum	Increased total bacterial load in severe COPD
Huang 2014 (110)	60 samples from 12 mild/moderate/severe COPD patients in a longitudinal study—sputum	Altered microbial communities with acute exacerbation, and with type of treatment for exacerbation (antibiotics, steroids or both)
Millares 2014 (111)	11 severe COPD; 5 COPD colonised by PA—sputum	Increased biodiversity during exacerbation in PA-infected sputum, to equal non-PA sputum

COPD, chronic obstructive pulmonary disease; PA, Pseudomonas aeruginosa.

Lung ageing

Ageing is an endogenous rather than exogenous factor, representing cumulative exposures to environmental factors over time. A wide range of phenotypes and biomarkers of ageing are currently being investigated in chronic diseases, including COPD (112,113). Examples of potential relevance to COPD progression include telomere shortening and sirtuins.

Telomeres are protective structures of repetitive sequence that stabilise the ends of chromosome by preserving genetic information and preventing DNA degradation (114,115). Telomere length varies between different cell types, tissues and individuals. Shortening of telomere repeats occurs naturally with cell division, with the shortened telomere ends eventually acting as a signal for apoptosis (116,117). For example, the reduction rate of telomere repeats in peripheral blood mononuclear cells is measured at approximately 84 bp per year, with an accompanying progressive decrease in telomerase activity, in healthy individuals under 40 years of age (118). Telomere length is also a predictor of years of healthy life in older persons (119). Because of this relationship with biological age, telomere length has been associated with ageing and age-related diseases such as COPD. Telomeres are shorter in peripheral blood leukocytes of COPD patients (120,121), particularly cigarette smokers (122,123), providing a common risk factor for accelerated ageing and replicative senescence in COPD.

Telomere length has been linked with lung function

in large population studies. A population study of 46,396 subjects (120) found an association between reduced leukocyte telomere length and COPD, and a weak correlation with lung function (FEV_1, FVC, FEV_1/FVC). A second study found circulating leukocyte telomere length was reduced in patients with COPD (n=934) compared to controls (n=15,846), and more strongly correlated with lung function in never smokers than in smokers (122). Telomeres were found to be relatively preserved in patients with AAT deficiency, compared to non-AAT-related, aged-matched COPD subjects, and there was good correlation between blood and lung telomere lengths (with blood being shorter on average) (124). Of prognostic importance, short leukocyte telomere length was associated with increased risk of all-cause mortality (HR 1.29), compared to longer telomeres, in 4,271 subjects with mild to moderate COPD in the Lung Health Study (125).

Sirtuins (SIRTs) are NAD^+-dependent deacetylases and are members of the silent information regulator 2 (Sir2) family (126), with seven homologues in man, SIRT1-7 (127). This family of enzymes is involved in gene silencing and several studies have demonstrated that SIRT1, an anti-inflammatory and anti-ageing protein, is decreased in the lungs of patients with COPD and peripheral blood mononuclear cells in COPD (128-130). Sirtuins also control resistance to oxidative stress and DNA repair (130) and SIRT1 activation reduces cigarette smoke-induced oxidative stress (131). MMP-9 is down regulated by SIRT1 and reduced levels of SIRT1 may cause structural changes in the lung tissue (126,132,133). Sirtuins were shown to be suppressed by cigarette smoking in the large airways of asymptomatic smokers and not in the small airways, whereas in COPD a greater suppression of sirtuin expression was seen in both the large and the small airways (127). Thus there is emerging evidence to suggest that a reduction in sirtuin expression is involved in accelerated lung ageing and pathogenesis of COPD (112).

Conclusions

COPD is a heterogeneous and complex chronic lung disease with extrapulmonary manifestations. Identification of clinically applicable biomarkers would help to screen for and diagnose COPD, monitor disease activity and progression, and guide response to therapy. Similar to other chronic diseases, the search for relevant biomarkers is certainly expanding rapidly in COPD. However, access to samples remains a major issue. Gene expression profiling

of lung tissue has identified genes whose expression differs in COPD according to severity, but markers derived from lung tissue are not routinely available for clinical disease monitoring, whereas sputum and blood are readily accessible. Biomarkers in blood, especially inflammatory markers such as fibrinogen, are associated with exacerbations and mortality in larger COPD cohort studies. Much more work is needed to assess blood and sputum biomarkers against disease progression outcomes in COPD. Emerging approaches to studying gene-environment interaction, which impacts on disease pathogenesis and progression in COPD, are providing promising leads for novel biomarkers. These include (I) sampling exhaled breath for VOCs and exhaled breath condensate for protein markers; (II) characterising responses of the lung to inhaled air pollutants; (III) applying knowledge of the lung microbiome to COPD phenotypes; and (IV) determining the significance of biomarkers of ageing such as telomere attrition. Overcoming methodological challenges in sampling and quality control will enable more robust yet easily accessible biomarkers to be developed and applied to optimise personalised medicine in patients with COPD.

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Bronchoscopic interventions for severe COPD

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Abstract: Chronic obstructive pulmonary disease (COPD) causes severe handicap among smokers. Most patients have to remain under continuous oxygen therapy at home. Moreover, respiratory infections are very common among these patients and vaccination is obligatory against influenza. Emphysema and bronchiectasis are observed with computed tomography (CT) and in several situations these parenchymal damages are responsible for pneumothorax in one case and pseudomonas aeroginosa infection. Novel mini-invasive techniques are used currently for emphysema treatments which are described extensively throughout our current work.

Keywords: Emphysema; valves; pneumothorax; chronic obstructive pulmonary disease (COPD)

Introduction

Chronic obstructive pulmonary disease (COPD) remains one of the most prevalent lung diseases in the world. Medical treatment options are limited and most only provide symptomatic improvements without mortality benefit. To date, supplemental oxygen for hypoxic patients and pulmonary rehabilitation are the only medical treatments that have been shown to improve mortality in severe COPD. Although surgical lung volume reduction (LVR) in severe COPD patients with heterogeneous emphysema has been available for some time, in 2003 a prospective randomized controlled trial clearly demonstrated that in specific patient categories, lung volume reduction surgery (LVRS) was superior to medical management and offered a mortality benefit (1). Despite this, many patients with

severe COPD are not candidates for this surgery due to other comorbid conditions. Minimally invasive therapies utilizing bronchoscopy are actively being studied with the ultimate goal of reproducing the advantages seen in surgical LVR with less risk and morbidity from the procedure. The bronchoscopic techniques that have been reported to date include unidirectional bronchial valves, biologic and polymer based techniques to occlude lung segments, small stents placed through airway walls to allow trapped air to exist (airway bypass stents), thermal/steam vapor ablation and endobronchial coils. Each of these techniques attempts to reproduce some of the benefits seen in LVRS with a less invasive and less morbid procedure. In this chapter we will discuss the concepts and limitations for each of these techniques.
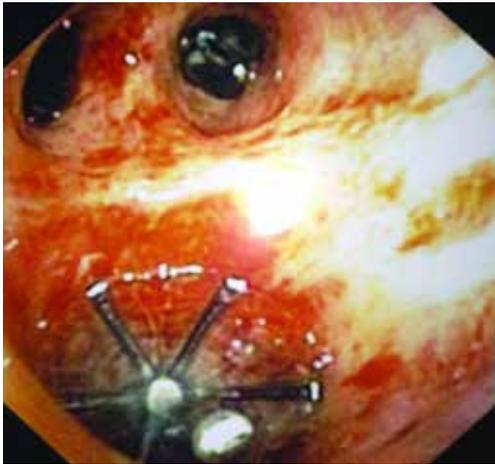
Figure 1 Three Spiration "intrabronchial" valve (IBV) occluding subsegmental airways.

Figure 2 Spiration "intrabronchial" valve (IBV) "umbrella" design.

Endobronchial one-way valves

Bronchoscopically placed endobronchial one way valves that occlude airways preventing air from entering severely emphysematous portions of lung yet allow air and mucus to exit have been proposed for the treatment of severe COPD (*Figure 1*). Initially the hypothesis was that the valves would induce atelectasis of the most severe emphysematous portions of the lung, thus decreasing the air trapping and hyperinflation of the treated lung. Other theoretical methods of action include the shunting or bypassing of severely emphysematous airways directing airflow to the more normal areas of lung to increase ventilation perfusion matching as well as decreasing the dynamic hyperinflation of the lungs during exertion by blocking these severely emphysematous airways. There are currently two types of valves that have been studied. The valves

are made by two companies, Spiration Inc. and Pulmonx (previously Emphasys Medical). Both valves are deployed bronchoscopically and are one-way valves. Spiration identifies its valve as an "intrabronchial" valve (IBV) and Pulmonx labels their valve an "endobronchial" valve (EBV) and is also referred to as the Zephyr EBV. The valves have unique designs to achieve the one way valve effect with the IBV being similar to an umbrella design (*Figure 2*) and the EBV appearing like a fish mouth facing the proximal airways that springs opens when air or mucus is exhaled from the distal airways but stays closed during all other times.

Zephyr EBV

The first pilot study for the endobronchial valves was published in March of 2003, by Toma *et al.* with eight patients with severe emphysema undergoing unilateral valve placement (2). All of these cases were patients with extremely severe disease, five of these patients had emphysema that was too severe for LVRS and the other three did not wish to undergo surgery. After unilateral valve placement, the median forced expiratory volume in one second (FEV_1) increased 34%, diffusing capacity of the lung for carbon monoxide (DLCO) increased 29%, and the computed tomography (CT) scans showed significant reduction in the volume of the treated lobe. Despite the extreme severity of emphysema in this series, complications were limited to two pneumothoracies with only one requiring drainage. This series was followed shortly by a similar study with 10 patients published in September 2003, by Snell *et al.* describing the implantation of EBVs in patients with severe emphysema and hyperinflation (3). Although no improvement in spirometric, functional or radiologic findings were seen at 30 days, no major complications were noted and the minor complications of COPD exacerbation (n=3), asymptomatic localized pneumothorax (n=1) and lower lobe pneumonia (n=1) were limited, thus adding to the feasibility and safety data for the procedure.

In 2004, Yim *et al.* (4) studied the feasibility, safety, and short-term efficacy of the EBVs in 21 patients. They showed significant improvement in spirometric measurement of FEV_1 and forced vital capacity (FVC) at 90 days and all functional parameters [6-minute walk, Short-Form Health Survey, St George's Respiratory Questionnaire (SGRQ), modified Medical Research Council (mMRC) at both 30 and 90 days]. Although a small study, the results were encouraging.

In 2006, Wan *et al.* (5) reported on the first multicenter

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experience (nine centers in seven countries) by retrospectively analyzing 98 cases from a prospective multicenter registry. In this cohort, there was a significant improvement in FEV_1, FVC, residual volume (RV), and 6-minute walk at 90 days. Eight point two percent had serious complications including one death. There was also an interesting trend toward improvement in those patients had an entire lobe treated compared to those with just one or two bronchopulmonary segments treated. They concluded that this was further evidence that bronchoscopic valve therapy could produce improvements similar to those seen in LVRS but patient selection needed further study.

In 2010, the Endobronchial Valve for Emphysema Palliation Trial (VENT) was published. In a 2:1 randomization scheme, 321 subjects with heterogeneous emphysema and severe COPD were enrolled and 220 received the EBV treatment. Primary endpoints were FEV_1 and 6-minute walk test (6MWT). Safety was assessed using a composite rate of six predetermined complications. The study showed small but statistically significant improvements in lung function (4.3% increase in FEV_1), exercise tolerance and quality of life but also showed a significant increase in complications such as COPD exacerbations, pneumonia and hemoptysis in the treated group. At 12 months, the rate of the complications composite was 10.3% in the EBV group versus 4.6% in the control group (P=0.17). Heterogeneity of emphysema and fissure completeness were analyzed using quantitative CT and an association was found suggesting a greater response to the treatment for subjects with greater heterogeneity of emphysema within their lungs and complete fissures (6,7). The European cohort of the VENT was analyzed and reported separately in 2012 by Herth et al. (8). Treatment group consisted of 111 subjects versus 60 in the medical management group. Unlike the initial VENT cohort results (6), the European cohort did not show a significant difference in rates of complications between the treatment and control group yet still had significant improvement in the FEV_1, cycle ergometry and SGRQ. Radiographic analysis of the CT scans demonstrated an association with better outcomes in those patients with complete fissures but no association with the degree of heterogeneity of emphysema.

Spiration IBV

In 2007, a multicenter trial using the Spiration IBV in 30 patients was published showing significant improvement in health-related quality of life metrics although no significant difference in physiologic and exercise outcomes (9). Five years later in 2012, Ninane et al. (10) reported a European multicentre, blinded, sham-controlled study evaluating the safety and effectiveness of the Spiration IBV for treatment of bilateral upper lobe emphysema. This trial was specifically designed to evaluate the treatment when performed without the goal of lobar atelectasis. Patients with upper lobe predominant severe emphysema were selected for the study. Seventy three enrolled with 37 randomized to the treatment arm and 36 to the sham treatment arm. Primary outcomes were the SGRQ and lobar volume shift measured by quantitative CT (increase in lower lobe volume in the setting of decreased upper lobe volume post treatment). A positive responder was required to have both a ≥4-point improvement in SGRQ and a lobar volume shift. Only 24% (8/37) of the treatment group and none in the sham group met criteria for a positive response. Overall, there was a significant difference in the lobar volume shift between groups. Both groups improved on the SGRQ. There were no differences between groups in adverse events.

Valve concept comparison

The Spiration IBV studies described above studied a bilateral upper lobe deployment strategy without the intent to produce lobar atelectasis but instead create a redistribution of ventilation to less emphysematous airways. The Zephyr EBV studies have concentrated primarily on the unilateral valve deployment with the goal of producing total lobar atelectasis. Recently, Eberhart et al. [2012] published a randomized trial directly comparing these two techniques. With 11 subjects in each arm, he demonstrated that the unilateral lobar atelectasis approach was significantly superior to a bilateral partially occlusive approach at 30 and 90 days when comparing improvements in pulmonary function tests (PFTs), 6-min walk distance (6MWD), dyspnea score as measured by the mMRC dyspnea score, and quality of life as measured by the SGRQ (11). Although the study was small, the evidence certainly suggests that producing total lobar collapse with the valves is more likely to result in clinical improvement for the patient. The existence of collateral ventilation in the lung was recognized by the work of Van Allen and others in 1931 (12). The evolution of the endobronchial valves and lessons learned from each trial have clearly demonstrated the importance of recognizing and mitigating collateral ventilation within the lungs. Research in devices that can identify areas of collateral circulation in an individual's lungs are actively being studied

Figure 3 PneumRx lung volume reduction coil (LVRC).

and initial studies have confirmed the ability to more effectively guide placement of endobronchial valves (13,14).

Lung volume reduction coils (LVRC)

LVRC is a technique for bronchoscopic treatment of severe COPD using a nitinol coil that is delivered to the mid lung airways under fluoroscopy in a sheath which keeps it straightened and then when the sheath is pulled back, the straightened coil returns to it's normal shape that resembles the seams on a baseball compressing the surrounding parenchyma and restoring elasticity to the emphysematous lung (*Figure 3*). This technique is unique because it is unaffected by collateral flow and appears to be effective on heterogeneous and homogeneous emphysema and is also independent of collateral ventilation. Since the proposed mechanism is restoring elasticity to the parenchyma from shortening of the airways, patients with large bullae are unlikely to benefit from the technique and have been excluded from current studies (15,16).

Results of the first pilot study using this device were published in 2010 by Herth *et al.* with 11 patients that showed safety and some evidence of efficacy. This pilot was followed by another trial in 2012 with 16 patients that demonstrated a significant improvement in SGRQ, FEV_1, FVC, RV, and 6MWT at 6 months with an acceptable safety profile (17). The first randomized controlled trial of

the device (RESET trial) was published by Shah *et al.* in 2013. With 47 patients total (23 LVRC, 24 control) there was a significant improvement in the primary endpoint of quality of life as measured on the SGRQ in the treatment group and no difference in the safety endpoints between the groups (18). While LVRC is one of the newer techniques for the bronchoscopic treatment of severe COPD, it has already distinguished itself as one of the more promising because of its safety profile and demonstrated ability to work in patients with both heterogeneous and homogeneous emphysema independently from the effects of collateral ventilation.

Airway bypass

Small endobronchial vents placed bronchoscopically through holes made directly in the airway wall to allow direct flow of trapped air in the emphysematous lung is referred to as airway bypass. Essentially the concept is that by creating holes in the airways and stenting them open with drug eluting stents, they will remain open and allow emphysematous areas of lung with trapped air to escape to the larger airways more easily. A doppler probe is used to interrogate the target site for the hole to ensure there is no vessel at that location. The exhale airway stents for emphysema (EASE) trial was a prospective randomized, double-blind, sham-controlled study published in 2011 that studied 315 subjects with homogenous emphysema and with severe hyperinflation documented by a ratio of RV to total lung capacity of ≥0.65 (19). Primary efficacy endpoint was at least a 12% increase in FVC and at least 1-point decrease in the mMRC dyspnea score from baseline. No difference between treatment and sham groups was seen in the efficacy or safety endpoints at 6 or 12 months. Initially the vents produced an improvement in lung function but the vents tended to become obstructed over time with granulation tissue or mucus and the improvements were not sustainable over time. The issue of vent obstruction was noted on earlier studies and the addition of drug eluting stents for the EASE trial was an attempt to prevent granulation obstruction of the vents. While the concept of airway bypass was confirmed, this technology does not appear to be effective in its current state.

Glue and biologics for bronchoscopic lung volume reduction

Adhesives have been used in the treatment and prevention

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of persistent air leaks complicating thoracic interventions. Fibrin sealant has been applied successfully via thoracoscopy in the ablation of persistent air leaks following tube thoracostomy or pulmonary resections (20). Additionally the use of surgical sealants has also been examined in the prevention of post-operative persistent air leaks. A recent meta-analysis of 13 studies comprising 1,335 patients concluded that the use of surgical sealants and buttressing decreases the risk of prolonged air leaks after pulmonary resections. The authors cautioned, however, that given the possibility of publication bias their interpretations should be used with caution and that further trials are warranted to confirm the effectiveness of this method (21).

Investigators have started to examine the effectiveness of bronchoscopic administration of adhesives in the treatment of advanced COPD. The application of a biologic sealant delivered bronchoscopically to sheep had been demonstrated to be effective in collapsing targeted areas of lung and inducing localized scar tissue formation and ultimately reduction of lung tissue volume (22). Using these principles Reilly and others conducted a phase I trial designed to evaluate the safety of biologic lung volume reduction in six patients with severe predominantly upper-lobe COPD based upon CT scan appearance. A biogel composed of fibrin and thrombin was instilled via a flexible bronchoscope through which a dual lumen catheter was placed. Three patients had two subsegments treated and three patients had four subsegments treated. There were no serious adverse effects and small improvements were observed in mean vital capacity, mean RV, mean RV/total lung capacity ratio (RV/TLC), mean 6MWT, and medical research council dyspnea (MRCD) score (23).

A larger phase II study was performed on patients with global initiative for chronic obstructive lung disease (GOLD) stage III and IV using the bronchoscopically delivered fibrin and thrombin hydrogel. Two different doses of the hydrogel were delivered into 50 patients with upper lobe predominant emphysema. All patients had eight subsegments targeted (four in each upper lobe) based upon the most diseased portion of the lung determined with pre-procedural high resolution CT scan (HRCT). Twenty-eight patients had 10 mL and 22 patients had 20 mL of the hydrogel applied to each targeted subsegment. There were no procedure related deaths, but there was an 8% incidence of serious cardiopulmonary adverse events. At 6 weeks of follow-up there were statistically significant improvements in FEV_1, FVC, RV/TLC, MRCD score, and health-related quality of life (HRQOL) score in both the low-dose (LD)

and high-dose (HD) groups. However, at 6 months only the HD group had sustained significant improvements in all physiologic outcome measures (24).

The two previous studies focused on patients with heterogeneous upper-lobe predominant emphysema. Another study examined the effect of the application of fibrin and thrombin hydrogel to patients with homogeneous emphysema and severe airflow obstruction defined as a FEV_1/FVC <70% and FEV_1 <45%. In this non-randomized study patients were selected to receive either the LD (n=8) or HD (n=17) dose as described in the previous study. The subsegments were chosen based upon the results of both CT and radionucleotide lung perfusion scanning and each patient had eight subsegments treated in the upper lobes or superior segments of the lower lobes. There were no procedure related deaths, though the majority of the patients experienced transient leukocytosis, fever, or malaise. There were also two COPD exacerbations in the LD group and three in the HD group. At 3 months of follow-up only the HD group had statistically significant improvement in the primary end-point, RV/TLC ratio (–6.9). There were also significant improvements in FEV_1, MRCD score, and SGRQ. The authors do note that the responses in patients with homogeneous disease were smaller than those with upper lobe heterogeneous disease reported in prior studies (25).

A synthetic polymer emphysematous lung sealant (ELS, AeriSeal®, Aeris Therapeutics, Woburn, MA) has recently been developed. The foam sealant is delivered via a single lumen catheter placed through the working channel of a flexible bronchoscope. Unlike the fibrin and thrombin hydrogel the foam sealant is not delivered as two separate components to the target site, but is rather mixed prior to instillation through the catheter. In a prospective, non-controlled study of 25 patients with heterogeneous emphysema the ELS was applied to up to six different subsegments during one or two treatment sessions. Similar to the patients that received the fibrin and thrombin hydrogel a transient inflammatory reaction was common. There were no treatment-associated mortalities, but there were ten acute or sub-acute COPD exacerbations. There were improvements from baseline at 24 weeks in FEV_1 (+10.0), FVC (+15.86), RV/TLC (–4.7), SGRQ score (–7.5 units) and 6MWT (+24.6 m), however, only the FVC has statistically significant improvements when corrected for multiple comparisons. The physiologic improvements in ELS therapy were greater in patients with GOLD stage III disease (26).

The ELS system was used in another small trial of 20

patients with advanced emphysema, 10 with upper lobe and 10 with homogeneous disease. The procedure was bilateral with two subsegments targeted in each upper lobe. The procedure was performed only with conscious sedation and was fairly short (approximately 15 minutes) with an average hospitalization of about 1 day. The primary end-point of reduction in upper lobe volume assessed by quantitative CT scan at 3 months was successful with a statistically significant reduction of 895 mL. There were additional physiologic improvements at 6 and 12 months in FEV_1, RV/TLC, DLCO, MRCD score, and SGRQ. One serious procedure complication occurred and there were seven significant respiratory adverse events during follow-up (27).

Similar to vapor therapy ELS has been demonstrated to lead to physiologic improvements despite the integrity of lung fissures. A report by Magnussen *et al.* summarized the results of three trials of patients that had undergone ELS treatment. Of the patients enrolled only 28 had complete data sets that allowed analysis. ELS therapy reduced lung volumes independent of interlobar fissure integrity in patients with heterogeneous upper-lobe emphysema. Additionally there were similar improvements in gas trapping, spirometry, functional capacity, and quality of life between those patients with complete and incomplete fissures (28).

Overall this data suggests that the application of adhesives in the treatment of emphysema has a safety profile better than surgical lung volume reduction and comparable to other methods of bronchoscopic lung volume reduction. There appears to be physiologic improvements in spirometry, functional ability, and quality of life. Both fibrin/thrombin hydrogels and a synthetic polymer have been used, but never compared in a single trial. This procedure leads to permanent subsegmental collapse and is not affected by fissure integrity. Despite the promise of several small studies, larger, randomized controlled trials are needed.

Vapor therapy for lung volume reduction

Vapor therapy for COPD is a new technology that delivers thermal energy in the form of heated water vapor to target subsegments of the lung. It is performed via a disposable catheter introduced through the working channel of a flexible bronchoscope and thus can be done in a minimally invasive manner to patients that are under moderate sedation. The InterVapor™ (Uptake Medical Corp., Tustin, CA) consists of a vapor generator and a flexible catheter.

Each procedure is specifically tailored to the individual based upon software analysis performed on a HRCT. The subsegments are targeted based upon the degree of heterogeneity and an energy dose of 5 to 10 calories per estimated gram of tissue is delivered to the selected subsegments. This thermal energy leads to an inflammatory response that ultimately causes contraction fibrosis and atelectasis with subsequent lung volume reduction. The results of this procedure are permanent unlike the placement of one-way endobronchial valves or coils that can theoretically be removed at a later date (29).

A feasibility study was performed in 11 patients with severe heterogeneous COPD. These patients underwent unilateral application of vapor therapy at five calories per estimated gram of tissue. Compared with the patients baseline data there were significant improvements in DLCO (16%) as well as improvement in the SGRQ at 6 months. There were, however, no significant improvements in FEV_1, FVC, RV or 6MWT during the same amount of follow-up. The improvement in SGRQ was reportedly superior to other methods of bronchoscopic lung volume reduction and the DLCO improvements were similar to those of surgical lung volume reduction. Side effects of the procedure included nausea, cough, hemoptysis, fatigue, and COPD exacerbations. There were seven COPD exacerbations in four patients three of which were judged to be infectious and four noninfectious in nature (19).

A larger study was performed in 44 patients with heterogeneous upper lobe emphysema. Once again the patients underwent unilateral treatment with the targets based upon prior CT scan evaluation. A calculated amount of energy of 10 calories per gram of tissue was applied to each targets lung segment for 3 to 10 seconds. Twenty-four patients were treated in the right upper lobe and 20 patients in the left upper lobe. At the 6-month post-procedure evaluation the patients had improvement in FEV_1 of 141 mL, and a reduction in RV of 406 mL. There was also an improvement in SGRQ of –14 units as well as 6MWT of 46.5 m. The most common adverse events were COPD exacerbations, which occurred in 11 patients most often within the first 30 days following treatment (30).

A follow-up study was performed in the same group at 12 months. The overall finding was that the treated patients continued to have improvement at the 1 year mark, although it was to a lesser degree that that seen at 6 months. The improvements in FEV_1 fell to 86 mL and RV to –303 mL. The SGRQ score was –11 units and the distance in 6MWT was only 18.5 m above pretreatment baseline.

On subgroup analysis it was found that improvements in lung function and exercise capability were similar at the 6-month evaluation, but greater at 12 months in those patients with GOLD stage IV disease compared to stage III. Twenty-three patients experienced 39 serious adverse events, 22 of which were respiratory. Two patients died during the follow-up period. One was secondary to end-stage lung disease at 67 days post-treatment and another occurred at 350 days post-treatment secondary to surgical complications following a lobectomy for aspergillus in the untreated lung (31).

Collateral circulation likely accounts for the failure of some patients to achieve atelectasis after large airway occlusion by endobronchial valves. This may be due to lack of fissure integrity. In a retrospective analysis of the study involving 44 patients discussed previously it was found that there was no to minimal association between lobar fissure integrity and the effectiveness of bronchoscopic thermal vapor ablation (BTVA). This is explained by the fact that areas of the lung that have undergone remodeling following contraction fibrosis are unlikely to re-inflate despite the presence of collateral ventilation. As such patients with incomplete fissures identified on HRCT may achieve more effective lung volume reduction than if they underwent large airway occlusion with a prosthetic device (32-51).

These small studies suggest that the application of steam to carefully selected sub segments of the lung in patients with heterogeneous upper lobe predominant emphysema may improve lung function, exercise tolerance and quality of life. These improvements may be more sustained in those patients with more severe disease. Unlike prosthetics such as endobronchial valves or coils the success of vapor therapy is independent of fissure integrity. Although promising, larger studies with prolonged follow-up are needed prior to wide application of this technology to the general population of patients with COPD (*Figures 1-3*).

Conclusions

Bronchoscopic interventions to treat severe COPD have made great progress over the last decade offering benefit to patients with few treatment options available. While trying to achieve the benefits seen LVRS with less morbidity and mortality, several diverse techniques described above have been developed. These various techniques have unique approaches that have uncovered some of the critical obstacles to success such as collateral ventilation and incomplete occlusion of the target lobe. As these techniques

advance, we may continue to see that in the wide spectrum of severe COPD patients, refinement of selection criteria for the respective techniques may have a significant impact on the results for the patient.

Acknowledgements

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Development of a self-treatment approach for patients with COPD and comorbidities: an ongoing learning process

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Background: Patient-initiated action plans are an important component of COPD self-management (SM) interventions. When integrated into SM interventions, these action plans have proven to be effective in reducing exacerbation severity, hospitalisations, and costs and in improving health status in patients with COPD without severe comorbidities. Because of overlap in symptoms, a self-treatment (ST) approach that focuses solely on traditional symptoms of COPD is inadequate for patients with COPD and comorbidities. The COPE-III SM intervention combines (I) patient-initiated action plans that are tailored to the individual's co-morbid disease(s), and (II) ongoing nurse support. In this paper we provide information regarding the integration of information from two previous COPD SM studies (COPE I and II) in the development of the current COPE-III ST approach.

Materials and methods: COPE-III ST materials include daily symptom diaries and action plans that take patient's common comorbidities [chronic heart failure (CHF), anxiety, depression, ischaemic heart disease (IHD), and diabetes] into account. The comorbid diary and action plans components were developed in collaboration with multiple disease-experts.

Results: Previous SM studies have highlighted some essential topics that need to be considered when developing a SM or ST approach: 'when to initiate ST', 'how to optimize materials and safety', and 'how to achieve behavioural change'. In the COPE-III study, ST is initiated after a significant change in symptoms. This is consistent with the COPE-II approach and was implemented because disease symptoms are often present even when patients are stable. We have tried to ensure patient safety by providing an easily accessible case-manager to patients throughout their involvement in the study. Furthermore, a psychologist has ensured the use of behavioural change techniques throughout the intervention.

Conclusions: We should continue to learn from our experiences with SM interventions to further optimize future SM and ST interventions. The use of materials that are suitable for different levels of patient literacy and the training of health care providers are other points of improvement.

Keywords: Pulmonary disease; chronic obstructive; self-care; comorbidity

Introduction

COPD is a leading cause of death and disability internationally (1) that affects approximately 1:10 adults in the developed world and is increasing in prevalence globally (2). High financial and social burdens have been associated with COPD in general (3,4) and COPD exacerbations in particular (5,6). COPD exacerbations, defined by episodes of acute deterioration in respiratory health (7), are also a major contributor to a step-wise worsening of quality of life in patients (7).

The latest Cochrane systematic review of COPD self-management (SM) has documented that COPD-specific SM interventions are associated with a reduction in hospital admissions (8). Patient-initiated action plans are an important component of SM interventions (8,9). When used appropriately, they can lead to accelerated initiation of appropriate treatment (10) and therefore reduce the exacerbation severity (11). When integrated into SM interventions, these action plans have proven to effectively reduce exacerbation severity, hospitalisations, and costs and improve health status (11-13).

Comorbidities are the rule rather than the exception in COPD (14,15). Over two-thirds of COPD patients (68.4%) suffer from at least one comorbidity, about 16% have at least two comorbid conditions (15), and one third of the COPD patients admitted to hospital have at least four coded comorbidities (16-18).

Because the symptoms of COPD and common co-occurring diseases overlap, a "one size fits all" approach that focuses solely on traditional symptoms of COPD is inadequate. For example, increased dyspnoea could relate to either a COPD exacerbation or a sudden deterioration of cardiovascular disease (e.g., heart failure) (19,20). Reliance on specifically designed for COPD symptoms and actions/treatments could therefore lead to the initiation of incorrect or delayed treatment.

The latter is highlighted by a recent study evaluating COPD-specific action plans in a COPD population with comorbidities (21). The study was terminated because of significantly higher mortality rates in the intervention group. No definite reason for this has emerged and the findings contrast positive outcomes of a comparable SM study (22). Nevertheless, the study (21) has resulted in controversy regarding the effectiveness of SM interventions, especially in patients with high burden of disease and co-morbidities (23). In these patients, SM interventions may be more challenging and not without risk of serious adverse events (23). It underlines the need for further evaluation of action plans in COPD patients with comorbidities.

In this paper we provide an insight into how we have used our experiences with our previous SM studies to develop a novel COPD self-treatment (ST) approach for patients with COPD and co-morbidities.

The COPE studies

During the last 15 years we have performed three large randomized controlled trials to explore effects of SM: the COPE-I (24), COPE-II (11,25), and COPE-III study (26). COPE stands for 'COPD study at Department of Pulmonology Enschede. Whereas the COPE-I and COPE-II study were performed in the Netherlands, COPE-III is a joint Dutch - Australian research project. Experiences from COPE-I and COPE-II have been used to develop the design for the COPE-III study. Details of all three COPE studies have been summarized in *Table 1*.

COPE-I

In the COPE-I study the effects of a comprehensive SM intervention were evaluated in 248 patients with moderate to severe COPD and no severe comorbidities (24). The intervention involved an individualized treatment plan that incorporated smoking cessation, optimisation of pulmonary status by pharmacotherapy, a standardised low-intensity exercise program, and a written ST action plan for COPD exacerbations that was based on symptom perception. If patients experienced an increase of respiratory symptoms and normally would have called their physician, they could start with a short course of oral prednisolone, and with onset of purulent sputum a course of antibiotics for which prescriptions were supplied (24). The study results showed no effects on quality of life and exercise capacity, and an increased number of exacerbations, defined as an increase of respiratory symptoms treated with prednisolone and/or antibiotics in the intervention group. However, because daily symptoms were not recorded in either study groups, it could not be clarified whether this meant that there was an over-treatment in the intervention group or an under-treatment in the control group (24).

COPE-II

In the COPE-II study (11), the extra value of a COPD SM component was evaluated. A group of patients who received

Table 1 Characteristics of the COPE-I, COPE-II, and COPE-III study

COPE study	Methods				Participants			Setting	SM intervention group		Primary outcome	Secondary outcomes
	Design	Intervention	Control	Follow-up (months)	Numbers	Major inclusion criteria	Major exclusion criteria		Session frequency and duration	Key topics		
I	RCT	SM, ST and non-standardized low-intensity exercise	Usual care	12	127 intervention; 121 control	COPD diagnosis; (ex) smoker; age: 40-75	Medical condition with low survival, serious psychiatric morbidity; other serious lung and cardiac disease	Outpatient clinic, exercise in private practices	4 weekly 2-hour group sessions and 1 follow-up 2-hour group session given 3 months after the 4th session. Professionals: respiratory nurse, physiotherapist	COPD knowledge, symptom recognition, COPD ST action plan, inhalation technique, exercise, relaxation, nutrition, breathlessness, energy conservation, communication and social relationships	HRQoL (SGRQ)	Symptoms and exacerbation frequency of COPD, self-confidence, 6MWT, lung function, doctor consultations, hospital admissions, days lost from work
II	RCT (2-by-2 factorial design)	SM, ST and standardized exercise	SM intervention and standardized exercise	24 (published: 12 months)	70 intervention; 72 control	COPD diagnosis; ≥3 exacerbations, and/or 1 respiratory hospitalization in previous 2 years; (ex) smoker; age 40-75	Medical condition with low survival or serious psychiatric morbidity; other serious lung or cardiac disease	Outpatient clinic, exercise in private practices	4 weekly 2-hour group sessions and 5 follow-up phone calls. Professionals: respiratory nurse, physiotherapist	COPD knowledge, symptom recognition, COPD ST action plan, inhalation technique, exercise, relaxation, nutrition, breathlessness, energy conservation, communication and social relationships	Exacerbation severity—daily symptom diary (exacerbation days and severity scores)	Exacerbation frequency, hospital admissions and days, courses of oral steroids and antibiotics, lung function, CRQ, CCQ, HADS, HR-QoL, health care utilisation, costs
III	RCT (international-multisite)	SM and ST	Usual care	12	Recruitment in progress	COPD diagnosis; ≥1 comorbidity: IHD, CHF, AD, DM; ≥3 exacerbations, and/or 1 respiratory hospitalisation in previous 2 years; age ≥40	Medical condition with low survival or serious psychiatric morbidity; other serious lung disease; low cognitive functions	Outpatient clinic	4 or 5 weekly sessions (2 individual 1-hour sessions; 2 or 3 two-hour group sessions) and 3 follow-up phone calls. Professionals: respiratory, cardiac, mental health and/or diabetes nurse	COPD and comorbidity knowledge, symptom recognition, ST action plan for COPD and comorbidities, inhalation technique, exercise, relaxation, nutrition, breathlessness, energy conservation	Number of COPD exacerbation days—daily symptom diaries	Exacerbation severity (symptom score), hospital admissions and days, number of CHF exacerbation days, comorbid symptom scores, courses of oral steroids and antibiotics, lung function, CRQ, ICFS, HADS, CSES, HR-QoL, SM behaviour and knowledge, self-confidence, satisfaction, adherence with ST protocol, health care utilisation, costs

6MWT, 6-minute walking test; AD, anxiety and/or depression; ATS, American Thoracic Society; CCQ, clinical COPD questionnaire; CHF, chronic heart failure; CRQ, chronic respiratory questionnaire; CSES, COPD self-efficacy scale; DM, diabetes mellitus; ER, emergency room; FEV₁, forced expiratory volume in one second; FVC, forced (expiratory) vital capacity; GOLD, global initiative for chronic obstructive lung disease; GP, general practitioner; HADS, hospital anxiety and depression scale; HR-QoL, health related quality of life; ICFS, identity-consequence fatigue score; IHD, ischaemic heart disease; RCT, randomised controlled trial; SGRQ, St George's respiratory questionnaire; SM, self-management; ST, self-treatment.

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a SM intervention that included specific training in ST (intervention group; n=70) was compared to a group of patients who received a similar SM intervention without this specific training (control group; n=72). The ST training component incorporated training in COPD symptom recognition (with the help of a daily symptom diary) and use of an action plan. The concerns from the COPE-I study regarding over-treatment in the intervention group were taken into consideration and the start of a COPD exacerbation was defined as 'a clear negative change in two major symptoms or one major and one minor symptom from baseline, for at least two consecutive days' [major symptoms: breathlessness, sputum production, sputum color; and minor symptoms: cough, wheeze, running nose, sore throat, and fever (>38.5 ℃) (27)] (11). This meant ST was only initiated 48 hours after an initial change in symptoms. Similar to the COPE-I study (24), COPE-II data showed a significantly higher use of courses of prednisolone and antibiotics in the ST group. However, the number of reported courses in the ST group was still lower than the actual number of exacerbations reported in the diaries, meaning that prednisolone was not used during every exacerbation. The final COPE-II study results therefore indicated that this approach did not lead to overtreatment, and indeed less COPD exacerbation days and lower costs occurred in the intervention group (11). In summary, the COPE-II study demonstrated that specific COPD ST training within a more general COPD SM training intervention leads to less exacerbation days and lower costs (11). However, these study results cannot be generalized to the large population of COPD patients with comorbidities.

COPE-III

The COPE-III SM intervention incorporates (I) patient-initiated action plans that are tailored to the individual's co-morbid disease(s) as well as their COPD, and (II) phone support from case-managers. The design of the COPE-III study, an international randomised controlled multi-centre trial, has previously been published and the intervention is currently under evaluation in both the Netherlands and Australia (26). Patient recruitment takes place in five hospitals [Netherlands: Enschede (Medisch Spectrum Twente) and Nijmegen (Canisius-Wilhelmina Ziekenhuis); Australia (Adelaide: Repatriation General Hospital, Flinders Medical Centre, Royal Adelaide Hospital)]. We expect that data collection will be completed by the end of 2015.

In the COPE-III study, we have incorporated at similar COPD ST component to that evaluated in the COPE-II study and combined this with action plans for common comorbidities [chronic heart failure (CHF), anxiety, depression, ischemic heart disease (IHD), and diabetes]. The comorbid action plan components have been developed in collaboration with multiple disease-experts (Cardiologist, Cardiac Nurse Practitioner, Endocrinologist, Psychiatrist, and Psychologist). In COPE-III, extensive patient training directed towards individualized materials is provided.

COPE-III ST approach

The COPE-III intervention involves a total of 8-9 hours of SM session time and several additional follow-up phone calls. A more specific description of the intervention has been provided in a previous paper (26). Because of the adjustment of intervention materials for comorbidities, materials are more complex than the ones used in previous two studies. It has therefore become necessary to deliver half the COPE-III training sessions individually instead of in a group and to allocate relatively more session time towards specific ST training compared to previous interventions. ST materials include a 'what are my usual symptoms' card, a daily symptom diary, and an action plan. During training in the use of these materials, hypothetical scenarios were incorporated to engage the patient in practicing the completion of the diaries and understanding appropriate use of the action plans.

As in previous studies, SM training is provided by case-managers (respiratory nurses). Patients are provided with information on how to contact the case-manager if they have any doubts or questions. Access to case-managers is available during office hours and patients are advised to contact their GP or Emergency Department during out of office hours. The case-manager also acts as a triage nurse when the cause of the change in symptoms is unclear and additional advice is necessary (26).

COPE-III ST materials

Even when stable, many patients with COPD experience symptoms of their respiratory disease and comorbidities, especially patients with moderate to severe disease (19). In the COPE-III intervention, the nurse and patient define together the patient's symptoms during a stable health state and summarize these findings in the patients' 'what are my usual symptoms' card. The patient is advised to use this card while completing the daily symptom diary and to

indicate whether symptoms have changed compared with their stable health state. So as in COPE-II (11), ST actions are linked to changes in symptoms rather than to existing symptoms. This approach requires that patients have skills and knowledge to recognize deterioration in their symptoms (28).

Patients are asked to complete the symptom diary that includes respiratory symptoms and relevant comorbid symptoms, every day. When patients do not experience deterioration in any of the predetermined symptoms listed in the diary during the last 24 hours, they are instructed to tick the box 'no change in symptoms' (indicating no further actions are required). Whenever they experience deterioration in any symptom listed in the diary, they are asked to report the level of change for each of the listed symptoms and if this change is of sufficient magnitude, consult their tailored action plan (26).

Besides the COPD component, all daily symptom diaries and action plans include one or more comorbid components in a pre-defined order: (I) CHF; (II) anxiety and/or depression (AD); (III) IHD; and (IV) diabetes. Diabetes action plans differ for patients with type 1, type 2 and prednisolone-induced diabetes. As such, there are 21 possible action plans that can be instigated.

Cardiac component
Similar action plans are provided for two cardiac comorbidities, IHD and CHF, in both Australia and the Netherlands.

For CHF three questions are included in the daily symptom diary regarding fluid retention (weight, swelling of ankles and abdomen, and waking up at night short of breath). According to the action plan, patients should increase/start their diuretic medication when they record 'a significant change' for two consecutive days for at least one of these questions. The expert team agreed that a change in weight of at least one kilogram in 24 hours should be considered a significant change. Patients are asked to contact the case-manager if symptoms do not decrease with diuretic therapy, or if they think they need more than the 3-day diuretic course as directed in the action plan. In the Netherlands patients are asked to contact their cardiac nurse directly.

A second CHF action plan component is included for safety reasons. Patients are asked to contact the case-manager (or cardiac nurse for Dutch patients) if they become more light-headed and/or dizzy. Consequently, the case-manager contacts the cardiac nurse to see if further actions are required (possible causes for these symptoms include rhythm disorder, over diuresis or a side effect of medication).

The existing action plan for IHD, developed by the 'National Heart Foundation of Australia', is being used with minor adjustments in lay-out (29).

Anxiety and depression
The action plan for anxiety and depression advises patients to commence relaxation exercises (which are practiced during the SM courses) if they experience increased AD. If symptoms do not improve after 5 days patients are asked to contact the case-manager (Dutch patients could directly contact the mental health worker). When necessary, their predefined 'plan' (e.g., seeing their GP to discuss their symptoms and management) is activated and/or a consult with a psychologist arranged.

Prior to inclusion patients are screened with the Hospital Anxiety and Depression Scale (HADS) (30). Patients with scores meeting recognized clinical cut-off points (exceeding 10 per subscale) of the HADS (30) are offered psychological counseling prior to the baseline measurement.

Although experiencing suicidal ideation is an exclusion criterion for the COPE-III study, standardised action plans are used if patients develop suicidal ideation during the study. For example, patients may contact nurses who conduct a risk assessment and patients are also provided with an emergency 24-hour phone number for specialised counselling for suicidal ideation.

Diabetes

Prednisolone treatment of COPD exacerbations increases blood glucose levels (BGLs), especially in patients with pre-existing diabetes. Hyperglycaemia in patients treated with prednisolone predominantly occurs between midday and midnight (31). Higher glucose concentrations are associated with increased mortality, morbidity and length of hospital stay during a COPD exacerbation (32,33).

Separate diabetes action plan components were developed for type 1, type 2 and prednisolone-induced diabetes. In contrast with the other comorbidities, the diabetes action plans are not linked to a change in 'diabetes' symptoms, but to the start of a COPD exacerbation. When taking prednisolone, patients are advised to check their BGL four times per day (before breakfast, lunch, dinner, and bed time). Extra training on blood glucose monitoring and insulin injections is then arranged with a diabetes nurse if required.

There are differences in the action plans for diabetes used in Australia and the Netherlands, in order to mimic as

much as possible usual care in both countries and simplify possible future implementation.

In Australia, patient management plans have been developed for two main groups of patients: (I) patients with diet-controlled diabetes or taking oral hypoglycaemic agents; and (II) patients already taking insulin. If patients record one BGL above 15 mmol/liter or two measurements above 10 mmol/liter, the action plan directs them to contact the case-manager who then contacts an endocrinologist. Patients who are not already taking insulin are taught to administer insulin isophane during COPD exacerbations, with dosing adjustments by an endocrinologist based on ongoing BGL recordings. Patients who are already taking insulin have their current insulin regimen doses adjusted by the endocrinologist.

In the Netherlands, patients with diet-controlled diabetes or taking oral hypoglycaemic agents are instructed to use insulin injections temporarily if they experience a high BGL (one BGL measurement above 15 mmol/liter or three measurements above 10 mmol/liter during a 24-hour period). Insulin dosing schedules are patient-fitted by the diabetes nurse and discussed during SM training. Patients have a tailored insulin dosing schedule (as advised by the diabetes nurse) or they are instructed to administer short-acting subcutaneous insulin using a sliding scale regimen.

Optimising of the COPE-III ST intervention

Prior to the start of the randomized controlled trial, the COPE-III ST intervention was tested in six patients with severe COPD to further optimize the intervention. Recruited patients were already included in an intensive nurse-led case-management program to which the COPE-III ST intervention was added. During the pilot, study nurses and patients were asked to provide frank feedback on the materials. During and after the pilot, significant adjustments were made to the ST materials. We have summarized an overview of these adjustments in *Table 2*. The intervention materials were adjusted to ensure that the intervention could be easily implemented in different health care systems.

Training of the health care providers

Both the COPE-I and COPE-II studies were extensively piloted (by groups of health care providers and patients). Besides optimising the intervention, the goal of these pilots was to train all health care providers in 'SM'. In addition, all involved health care providers in the COPE-III study

attended a half day course regarding the guidance of group sessions. The content of this course included discussion of behavioural change techniques that were embedded in the SM sessions: components of education, training, modelling, and enablement, which target desirable and specific behaviours including individualised diary use, patient recognition of deterioration in symptoms, and the correct and timely use of an action plan (26). Ongoing, regular follow-up meetings (approximately once a month) were planned with the health care providers involved.

The COPE-III study was also extensively piloted by patients and health care providers. The education in comorbidities was provided by disease experts in both countries (approximately 2-3 hours per comorbidity) and predominantly directed towards triaging of problems that could occur in these complicated COPD patients. Overlap in disease symptoms was discussed intensively. The training in SM and behaviour change principles was provided by an Australian psychologist during a 2-hour group meeting. This meeting was recorded, so it could also be viewed by the study nurse in the Netherlands.

Separate training in the diaries and action plans was provided by the study investigators in both countries (approximately 4 hours), with frequent follow-up meetings, that were especially important during the first year of the study.

Discussion

The COPE-III study is focused on treatment of COPD and common comorbid diseases. The intervention was developed and adjusted by using experiences and knowledge learnt from two previous COPE studies and by a pilot study. Although the action plans used in COPE-III are established and cannot be changed during evaluation, we are aware that we can continue learning from our experiences with COPD ST.

In the COPE-III study, we are attempting to deal with two of the most important lingering issues within ST, namely dealing with comorbidities and ensuring patient safety. We believe that a 'one size fits all' approach that focuses solely on traditional symptoms of COPD is inadequate and in fact, potentially dangerous in patients with (numerous and severe) comorbidities. This was the rationale underpinning the COPE-III approach. We have tried to optimize patient safety by ensuring a case-manager who is accessible to patients throughout the study. This is emphasized during patient training and highlighted on all ST materials. We also incorporated fallback procedures into the action plans, such as contacting usual health care

Table 2 Summary of adjustments of self-treatment materials (usual symptom cards, symptom diary, action plan, course material) as a result of the pilot study

Aims	Documents	Adjustments
Simplification of education material	All materials	Comorbidity components are colour coded and numbered
	Symptom diary	Reduction of numbers of items by combining the 'minor respiratory symptoms' in one question
	All materials	Remove medical jargon and simplify text
	Symptom diary	Make more clear that the action plan needs to be consulted by using red 'marked' boxes for a change that is 'significantly more than usual'
	All materials	Consistency in wording
	All materials	Consistency in the order in which comorbidities are addressed
Better discrimination between breathlessness due to COPD or due to IHD and CHF	Usual symptom cards	IHD item: record what patients normally use as IHD medication (e.g., a spray or a tablet)
	Symptom diary	IHD item: use of 'sudden change in your breathing' instead of just 'short of breath'
	Action plan	Inclusion of a final box with the comment: 'If you have been significantly more breathless than usual (marked red boxes) for at least 2 days in a row but you did not tick any red boxes for other symptoms: please contact the study office'
	Course material	Extensively discussion of breathlessness by working through scenarios
Stimulating patients to go through the complete action plan	Action plan	Insert a clear message after every box in the action plan to go to the next part of the action plan
	Course material	Practising with the action plan and underlining to read through the complete action plan
Increasing of safety of the ST approach	Symptom diary, action plan, course material	Making clear that patients can always contact the study nurse if uncertain or having questions
	Action plan	Adding a final box to the action plan with the following messages: ➢ Contact the study office if you have been significantly more breathless than usual (ticked red boxes) for at least two days in a row but you did not tick any red boxes for other symptoms ➢ Contact your GP if you have a fever (more than 38.5 ℃) for at least 2 days in a row but you did not tick any red boxes for other symptoms ➢ Please check the action plan tomorrow again and remember: you can always contact the study office during office hours if you have any doubts or questions 'phone number' (Monday-Friday: 8.00 am-4.30 pm; excluding Public Holidays) ➢ If you require assistance during out of office hours: please contact your GP or Emergency Department

IHD, ischemic heart disease; CHF, chronic heart failure; ST, self-treatment; GP, general practitioner.

providers for unresolved or worsening breathlessness or fever (see *Table 2*). The safety of the study is monitored by a Data and Safety Monitoring Board.

Another recommendation is that ST approaches have to be included in a formal SM training intervention (10) that includes behavioural change techniques (9) and is tailored to the patient's individual needs (9). The COPE-III intervention meets all of these criteria. Behavioural change techniques are included in an extensive patient training intervention (e.g., education, training, modeling, individualised action plans, behavioural enablement, individualised goal setting, and feedback on behaviour). Although ST of co-morbidities is patient-tailored, the content of the SM training is part of an intervention with set components (e.g., disease education, relaxation, and breathing techniques). In COPE-III we have utilised a ST approach that provides appropriate tools, training in necessary skills, and the possibility to incorporate the approach in existing health care support systems (9).

Additionally, health literacy of patients should also be taken into account. Literature suggests that only a third of patients with low literacy are able to comply with simple written instruction such as 'Take two tablets by mouth twice daily' (34). We are acutely aware that our ST materials are much more complicated than this instruction, and we concede that SM is not an approach that would be suitable

for all patients with chronic diseases like COPD. However, lessons were learnt during the pilot study and the patient materials were simplified. Although we exclude patients who are non-literate and those assessed as having an impaired cognitive function (26), we have not excluded people with low health literacy in any of the COPE studies.

For ST of COPD exacerbations it is also important to keep in mind that patients should be able to use their action plans regularly. If their symptoms are not varying with some frequency, amounting to repeated exacerbations, there are no opportunities for them to refer to their action plan and therefore learn from or receive feedback on their actions. In COPE-II and COPE-III it was therefore decided to include only frequently exacerbating COPD patients (patients who had at least three exacerbations or one respiratory related hospitalization in 2-year previous to inclusion).

At present there is no general agreement on the specifics of training health care providers to deliver optimal SM interventions, although experts agree that training of health care providers is crucial. In preparation for COPE-III, a psychologist was asked to provide a discussion session regarding behavioural change techniques that could be included in the COPE-III intervention. As this is an important aspect of SM, additional follow-up meetings were organized to discuss behavioural change techniques.

Finally, little is known about the factors influencing the success and failure of SM interventions, although understanding is growing as we acknowledge the intricacies of human behaviour and what drives behaviour change. Perhaps even less is known of the factors influencing the success and failure of ST interventions, and further studies will hopefully shed more light on this in the near future.

Acknowledgements

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Diagnosis and early detection of COPD using spirometry

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Abstract: The standard respiratory function test for case detection of chronic obstructive pulmonary disease (COPD) is spirometry. The criterion for diagnosis defined in guidelines is based on the FEV_1/FVC ratio forced expiratory ratio (FER) and its severity is based on forced expiratory volume in one second (FEV_1) from measurements obtained during maximal forced expiratory manoeuvres. Spirometry is a safe and practical procedure, and when conducted by a trained operator using a spirometer that provides quality feedback, the majority of patients can be coached to provide acceptable and repeatable results. This allows potentially wide application of testing to improve recognition and diagnosis of COPD, such as for case finding in primary care. However, COPD remains substantially under diagnosed in primary care and a major reason for this is underuse of spirometry. The presence of symptoms is not a reliable indicator of disease and diagnosis is often delayed until more severe airflow obstruction is present. Early diagnosis is worthwhile, as it allows risk factors for COPD such as smoking to be addressed promptly and treatment optimised. Paradoxically, investigation of the patho-physiology in COPD has shown that extensive small airway disease exists before it is detectable with conventional spirometric indices, and methods to detect airway disease earlier using the flow-volume curve are discussed.

Keywords: Spirometry; chronic obstructive pulmonary disease (COPD); case finding; flow-volume curve

Pathology of chronic obstructive pulmonary disease (COPD)

Relatively early research in the 1950s and 1960s into what was by then recognized as a smoking-related disease (1) focused on pathology, and especially tissue remodeling changes in the airways and lungs. It was observed that throughout the airways there was some element of inflammation, but sub-mucosal mucous gland hyperplasia, epithelial goblet cell hyperplasia and epithelial squamous metaplasia were prominent. The characteristic lung lesion was usually peri-bronchial, centri-lobular parenchymal destruction, termed emphysema (2-4). An important conclusion from the detailed pathological analysis of this epoch was that the airway pathological component in COPD was universal and generalized, while emphysema usually developed later, perhaps as a secondary phenomenon, and only in some individuals but by no means all. This is different

from the diffuse primary pan-acinar emphysema that occurs in the younger-onset alpha-1 anti-trypsin (anti-proteinase) deficiency lung disease, for example (5).

The next research epoch involved innovative physiological laboratory work in the late 1960s into the 1970s, which defined the obstructive consequences of smoking-related airway disease and the anatomical site of increased airway resistance that ultimately lead to symptoms (6,7). From this work, construction of a series of iso-volume pressure-flow curves gave rise to development of the now widely used flow-volume curve, but then without the sophisticated, sensitive and computerized equipment now available, and which we will be discussing later in some detail. However, even by that time and using the relatively crude bellows-based spirometer, the standard measure for defining airway obstruction had been specified as a reduction in the ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC), the forced expiratory ratio

(FER); indeed in that regard little has changed over the last fifty years or so, in spite of improved understanding of physiology. Paradoxically, the seminal work of Macklem and others in clinical physiology showed that the first change in spirometry in COPD was actually a reduction in FVC due to air trapping, rather than a change in FEV_1 (8). Importantly, they showed that this in turn is caused by fixed small airway narrowing, in airways less than 2 mm internal diameter. To demonstrate this, they used flow-volume studies in patients and volunteers with gases of different densities, and also measured flow resistance in different parts of the airway with retrograde catheters in resected lungs. Normal small airways have low resistance to air flow but this is markedly increased in COPD (9-11). In contrast, in asthma the main pattern is one of non-uniformly distributed larger airways obstruction, except in older asthmatics and those that smoke in whom a peripheral distribution of resistive change was common, similar to COPD.

Notably, it was shown in this epoch of physiological research, that there could be a great deal of peripheral increase in flow resistance before there was any indication on traditional spirometric measures. Patho-physiological correlation studies followed, indicating that in small airways in COPD there is indeed narrowing due to wall thickening, fibrosis and indeed airway obliteration (10,12,13). This was a new and startling insight, which is now confirmed by more sophisticated methodology (14), that there can be extensive small airway disease, damage and obliteration before it is detectable with conventional spirometric tests.

Over the years, new physiologic methods were developed to try and pick up these early small airway changes in smokers before overt COPD, defined by the FER emerged. However none was robust or practical enough at the concurrent stage of technological development to be suitable for clinical laboratory or medical office use. Such attempts continue with increasingly sophisticated techniques, and this is dealt with in a separate article in this volume; the FER remains the standard. In this article, we will review and discuss how useful in clinical practice this standard measure is, what we know about its pitfalls in clinical application and especially in primary care practice. We will also look anew at how the use of all the information available in the current standard flow-volume curve, which is now routinely obtained at the time of FER measurement but largely ignored, can potentially be harnessed and give a better overview of the status of the airways. This might contribute to recognizing early physiological impairment in smokers, perhaps as an alternative to the need to develop more expensive and complex tests.

Epidemiology and prevalence of COPD

The prevalence of COPD varies across countries; accurate estimates based on standardised population-based sampling of adults aged 40 and over in 12 sites in the burden of obstructive lung disease (BOLD) survey indicated an overall COPD prevalence (GOLD stage II or higher, FEV_1 <80% predicted) (15) of 10.1% (SE 4.8) (16). Prevalence increased with age and smoking history, but other factors were also thought to be important in explaining the variation. In the Australian BOLD survey conducted in six centres, prevalence was 7.5% (95% CI, 5.7-9.4%) overall, but was greater among those aged above 75 years at 29.2% (95% CI, 18.1-40.2%) (17). The Australian survey showed large variations between centres the causes of which are being investigated (unpublished data).

Estimates of the population attributable fraction of tobacco smoking as a cause of COPD vary by age and population setting (18), although more recent estimates in those aged 30-69 years, 54% for men and 24% for women, are probably accurate and less than the widely quoted 80-90% in the 1984 US Surgeon General Report (19). Attributable fractions are higher in industrialized countries than developing countries (18), and other risk factors are also important, including exposure to biomass smoke, occupational exposures to dust and fumes, history of pulmonary tuberculosis, outdoor air pollution, and poor socioeconomic status (20) or chronic asthma (21). However smoking remains the most important cause of COPD in western countries. Around 50% of smokers eventually develop COPD, although the risk falls by about half following smoking cessation (19).

Diagnosis of COPD

As discussed previously, spirometry is accepted as the diagnostic test to assess airflow obstruction and classify severity of disease, based on specific cut points for FER (FEV_1/FVC <0.7 after bronchodilator) and FEV_1 (mild ≥80% predicted, moderate 50-80%, severe 30-49% predicted, very severe <30% predicted) (15). FEV_1 normally decreases with age, and the rate of fall is an important spirometric indicator of disease progression in COPD. In healthy non-smoking adults the decrease is about 30 mL/year with an upper limit of about 50 mL/year (22-24); a decrease greater than this is considered abnormally rapid. There is

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debate on the use of a single fixed cut-off for FER to confirm the presence of airflow obstruction in COPD, because the lower limit of normal for FER decreases with age (25). Thus, this may misclassify some older patients as having COPD (26). Similarly, basing the classification of COPD on FEV_1 as percentage predicted may misclassify patients especially the elderly and those in global initiative for chronic obstructive lung disease (GOLD) stages I and II (27). It has been proposed that classification should be based on a lower limit of normal (LLN) i.e., more than 1.64·SD below the predicted level (5th percentile) (28), although international guidelines still recommend use of the fixed FER for diagnosis (15).

Maximum flow achieved during forced expiration decreases progressively as lung volume falls and is most evident in the expiratory flow-volume curve where flow is plotted as a function of volume. Although flow and volume are complex biological signals, the curve is highly repeatable in both healthy and obstructed individuals and the shape of the curve can be helpful as it reflects the underlying mechanics limiting maximal flow. In healthy younger adults the shape of the flow-volume curve usually approximates a straight-sided triangle with maximum flows decreasing linearly with lung volume. In people with obstructive lung disease key physiologic features of the flow-volume curve are reduced expiratory flows in proportion to disease severity and the presence of a concavity in the descending limb; the latter indicating an abnormal decrease in maximal flow as lung volume falls.

Flow measurements derived from spirometry such as the forced expiratory flow over the middle half of the FVC ($FEF_{25-75\%}$) and forced expiratory flow at 75% of the FVC ($FEF_{75\%}$) may be more specific to small airway function, particularly in the presence of a normal FEV_1, but they have not proved particularly helpful because they are dependent on the measurement of FVC, lack the repeatability of FEV_1, have a wide normal range, and are reduced in the presence of narrowing occurring in proximal airways (29,30).

COPD recognition and detection

However, in spite of spirometric standards for diagnosis, a high proportion of COPD in the community remains undiagnosed; estimates of non-diagnosis in the 1990's were 66% in the US (31) to 78% in Spain (32). Under-recognition is related to the severity of airflow obstruction; 50% of those with FEV_1 <40% predicted reported a physician diagnosis of COPD, but only 19% of those with

FEV_1 between 60-79% predicted in the Obstructive Lung Disease in Northern Sweden study (33). More recently, only 5.2% of BOLD population survey participants in Australia reported having been diagnosed with COPD compared to the 7.5% prevalence detected (17). Undetected COPD or asthma is common in primary care; over half those aged between 25-70 years in general practices in the Netherlands had symptoms or signs (34). There is also consistent evidence of misclassification of COPD in general practice. Substantial misclassification (31% and 42%) based on practice records COPD diagnosis was found in two studies in Australia (35,36). This probably relates to the diagnosis not being based on objective spirometry testing criteria.

Increased detection of COPD may result from a community-based screening programme; 27% of participants aged over 40 years had airflow obstruction based on FER <85% predicted in outpatient clinics in Poland (37). However such screening has not been widely implemented; a US Preventive Services Task Force assessment of the evidence did not recommend screening with spirometry and concluded with moderate certainty that there was no net benefit (38).

A more cost effective strategy using opportunistic case finding in primary care based on the presence of risk factors (age and smoking) and symptoms is recommended in the UK Update Guideline on COPD (39). A substantial amount of undiagnosed clinically significant COPD was demonstrated in the Health Survey for England 1995-6 (40). In over half these cases of unrecognized COPD management guidelines recommend treatment, either with combination inhaled corticosteroid/long-acting beta agonist or anticholinergic inhaler to reduce hospitalisation and mortality, or pulmonary rehabilitation to improve quality of life (40). Case finding can be effective when conducted opportunistically for patients attending general practice for any reason (41), compared with only a small improvement for 'targeted' case finding using pre-attendance practice register searches and mail out invitations to selected patients (42).

In many health systems, primary care provides the most accessible and most frequently accessed health care and efforts to increase recognition and diagnosis of COPD have mainly focussed on general practice (43,44). Spirometry testing should focus on those at risk particularly from smoking; thus spirometry was able to detect unrecognised airflow obstruction (FEV_1 <80% predicted) in 22% of current smokers aged 35 to 70 years with at least one typical COPD symptom in the Netherlands (41). The proportion of COPD of at least GOLD grade II (FEV_1 <80% predicted) in smokers aged over 40 in general practices

varies, from 25% in a Canadian study (45) to 47% in a study in Belgium (46), with only around a third in both already having a COPD diagnosis.

An alternative approach is to base spirometry testing on respiratory-relevant symptom screening using a questionnaire (47), with the cut-off score for subsequent spirometry chosen to maximise sensitivity and specificity (48). In this way, using a COPD screening questionnaire (48) and a cut-off score of 17 or above (range, 0-40) in patients over 40 years attending general practice in Greece, the sensitivity for new COPD diagnosis was 93% but specificity was only 39% (49). Simple inexpensive hand-held spirometers are available for use in general practice; they display FEV_1, forced expiratory volume in 6 seconds (FEV_6) as a surrogate for FVC, and the ratio FEV_1/FEV_6. Applying a cut off ratio FEV_1/FEV_6 <0.7 after bronchodilator in the same study in Greek general practices, increased the specificity to 94% with sensitivity of 80% for COPD diagnosis (49).

A further refinement for identifying COPD in general practice is to combine a COPD symptom questionnaire with measurement of FEV_1/FEV_6 ratio. In the Greek study quoted above, the combination of the questionnaire with its high negative predictive value and the hand-held spirometer with its high positive predictive value had a sensitivity of 74% and specificity of 97% for COPD diagnosis, while the negative predictive value was 95% and positive predictive value was 82% (49). In a scenario when individuals at risk of COPD in primary care were screened with a hand-held spirometer before full spirometry testing, a cut off point corresponding to FEV_1/FEV_6 <0.75 was found to offer optimal sensitivity (81%) and specificity (71%) for diagnosis in current and former smokers aged over 50 years (50). Linking symptom screening to case finding for COPD is ideal if the intention is to commence treatment in symptomatic individuals, but this approach is less suitable if the aim is to reduce end-organ disease.

Symptoms and a diagnosis of COPD

The place of symptoms in the diagnostic criteria for COPD has been debated (51) and there is some inconsistency between GOLD (15) and NICE (39) guidelines, with NICE advising not diagnosing COPD in the absence of symptoms in patients with mild airflow obstruction (FER <0.7, FEV_1 >80% predicted) (52). However, there is substantial evidence that reported symptoms are unreliable for diagnosis, although in general the symptom burden in COPD increases with severity of airflow obstruction. There

is wide variation in the degree of breathlessness, health status and exercise capacity within GOLD stages; thus even when airflow obstruction is severe in COPD, some people do not report symptoms or exercise limitation (53). There is also under-presentation by patients with potential chronic respiratory disease who do not raise respiratory symptoms with their general practitioner; 46% of patients with spirometrically confirmed COPD had not paid a single visit for respiratory health problems during a 10-year observational study in the Netherlands (54). Patients may attribute their symptoms to ageing and attribute multi-casual explanations that lessen the importance of obtaining a diagnosis (55). On the other hand, respiratory symptoms typical of COPD may be noted in practice records for long periods prior to diagnosis (56), with varying attitudes and degrees of vigilance among general practitioners to early diagnosis (56,57). Thus diagnosis of COPD may be delayed and indeed often does not occur until an acute exacerbation results in admission and hospital-based diagnosis (57).

Early diagnosis

Early diagnosis is a contentious issue, but it optimises the opportunities to prevent worsening of disease and prevention of comorbidities. Guidelines for COPD emphasise that it is a multi-system disease requiring a multidimensional approach to treatment (52). There is a strong emphasis on smoking cessation in both NICE (39) and GOLD (15) guidelines as the intervention with the greatest capacity to influence the natural history of COPD (58). Although a review in 2007 of randomised controlled studies on the value of spirometry itself as a motivational tool to increase smoking cessation was inconclusive (59), telling smokers their 'lung age' based on spirometry testing increased 12 months sustained quitting by over 7%, irrespective of the actual deficit in 'lung age' (60).

An increased risk of lung cancer in COPD was found in a long term US observational study in moderate or severe COPD (61) and in a case control study in lung cancer (62). The increased risk with COPD is present even when allowance is made for cigarette smoking history.

Similarly, the association of reduced FEV_1 with increased overall mortality has been recognized in studies in non-smokers (63) and smokers, with the effect of reduced FEV_1 independent of smoking history (64). The potential importance of the FVC was highlighted in a USA general population cohort without chronic respiratory diagnoses or persistent respiratory symptoms, in which survival was associated with

higher FVC in both men and women after adjustment for smoking and demographic factors (65). Such associations underlie the need for an earlier awareness of abnormality on spirometry as a part of a general health screening approach, such as was taken in cardiovascular disease to reduce the high burden of mortality that existed 40 years ago (66).

Value of current diagnostic tools for COPD: spirometry

Spirometry is a safe, practical and reproducible maximum breathing test that can be used in primary care to objectively determine the ventilatory capacity of the lungs. As already emphasised earlier in this article, it is the 'gold standard' for detecting and quantifying airflow obstruction (15) and as discussed, is the core component of clinical guidelines for the diagnosis and management of COPD (67). The test is relatively quick to perform, well tolerated by most patients and the results are immediately available to clinician. It is important to appreciate that the clinical value of spirometry is critically dependent on the correct operation and accuracy of the spirometer, performance of the correct maximal breathing manoeuvre, selection of the best test results to use and correct interpretation. When a trained and experienced operator using modern equipment conducts the test, at least 90% of adults are able to provide acceptable and repeatable results (68). In the primary care setting the rate is lower but can still be reasonable at about 80%, especially when the spirometer grades each test and provides feedback relating to test quality (69).

Development of spirometry

A spirometer is a medical device that allows measurement of how much air is expelled and how quickly the lungs can be emptied, in a maximal expiration from full inflation. Modern spirometry has its origins in the 1840's when the English surgeon, John Hutchinson, developed the spirometer and described the measurement of slow vital capacity as a means of detecting lung disease (70). One hundred years later Tiffeneau and Pinelli from France revolutionised spirometry by describing the forced expiratory timed spirogram and introducing an obstructive index, the ratio FEV_1/inspiratory vital capacity (IVC) which is still used today, albeit with IVC most commonly replaced with FVC (71) or expiratory vital capacity (72). It was only a few years later in 1960 that the American physiologists, Fry and Hyatt, in a landmark study of lung mechanics, replotted

the data contained in the timed spirogram in the form of the flow-volume curve (73) which is now universally accepted as the preferred method of graphically displaying spirometric data. The flow-volume curve is now available in almost all commercially available spirometers and is displayed in real-time as the patient performs the test.

Modern spirometers

Almost all modern spirometers utilise a sensitive real-time flow sensor to directly measure respired flow and obtain volume by electronic or numerical integration. Manual volume-displacement spirometers are still in limited use, especially in primary care (74), such as the iconic wedge bellows Vitalograph which over many decades has played a very significant role in popularising the measurement and application of spirometry beyond the expert laboratory, but this genre of spirometer usually lacks portability, is difficult to clean and disinfect, can be difficult to calibrate and requires spirometric variables to be calculated manually and does not produce the flow-volume curve.

There are many spirometers on the market today and most are robust, portable, accurate and reliable and specifically designed for use in either a lung function laboratory or a physician's office (74). Most, if not all, modern spirometers meet minimum international performance standards and validation procedures that were developed jointly by the American Thoracic Society and European Respiratory Society (75). These include meeting accuracy requirements for volume, flow and time signals using specifically developed test signals, and applying the back-extrapolation technique to identify both sluggish starts to the blow and the zero time point from which timed volumes such as FEV_1 are calculated. Modern spirometers also have the added advantages of infection control, automatic calculation of all lung function indices including correction for temperature, pressure and water-saturation conditions. Many will also provide immediate computer-generated feedback to the operator on the test quality and repeatability as well as real-time graphical display of the spirogram and flow-volume curve, will select the best results to report, calculate normal reference values including the lower limit of normal, and can automatically upload results to medical records.

Primary care spirometry

Spirometry is commonly performed outside the lung function laboratory. A survey of primary care practices in

Australia found that 64% owned a spirometer with almost 70% performing at least one test per week mainly for the diagnosis and management of asthma and COPD (76). The high spirometer ownership was not surprising given that a large number of patients with lung disease are first seen and subsequently managed in primary care.

Opinion is divided as to whether the quality of spirometry performed outside expert laboratories meets adequate minimum standards (75) with the potential for high rates of misclassification, especially when the results are near the lower limit of normal (69,77-79). The measurement of spirometry requires a motivated and enthusiastic operator to coach the patient to perform a number of very rigorous maximally forced and sustained breathing manoeuvres (80). It is not surprising therefore that unlike most other medical tests such as the measurement of blood pressure and the electrocardiogram, the quality of spirometry tests are crucially dependent on the operator and cooperation of the patient and thus spirometry performed in primary care is often of poor quality (81). Although the key to obtaining quality spirometry is attending a comprehensive training course, the importance of testing experience cannot be overstated and may well be the most important factor.

The concave pattern on flow-volume curve

Current guideline criteria for airway obstruction and its severity essentially rely on just two variables FEV_1 and FVC, and their ratio the FER. Although these variables have played an important role in developing our understanding of the mechanisms and functional effects of COPD, we have emphasised that they are relatively insensitive to early obstructive small airway pathology, because these cause FVC to fall first (8) with initial preservation of the FER. Spirometry has thus been of limited use as a screening tool for early disease; this is disappointing as it is the most practical and widely performed test of lung health and should therefore be ideal to screen for early disease. We present a case that relying solely on the FEV_1 and FER potentially misses information contained in the whole flow-volume curve, particularly the concave pattern, which may provide greater sensitivity in detecting and monitoring early disease.

The development of concavity in the descending limb of the maximum expiratory flow-volume curve is a recognised feature of airflow obstruction, with greater concavity reflecting increased obstruction, and the first indication of a concavity is frequently seen in the tail of the curve (30,72). This is explicitly acknowledged in the ATS/ERS statement

on the interpretation of lung function (82), but has largely been ignored in practice because none of the measurements taken currently to reflect this concavity are robust enough.

The functional information provided by the FEV_1 is necessarily limited to the first second of the forced expiratory manoeuvre when the lung is relatively fully inflated and the small airways exposed to significant distending forces. This means that in older people with a normal FER, as much as 40% of the flow-volume curve is not assessed, all in the terminal portion of the curve, and a greater proportion in people with airflow obstruction. In contrast, the concave pattern seen in people with airflow obstruction is not limited to the first second but often extends over most of the curve, reflecting a global pattern of airway dysfunction. In early airflow obstruction, when the FEV_1 is normal, a concavity is often present and may well be mostly confined to the terminal portion of the curve where lung volume is relatively low and the distending forces on the small airways are significantly reduced, resulting in a higher peripheral airway resistance and non-uniform emptying in peripheral lung regions. It is notable that the latter may well be the major spirometric defect signalling early disease, and requires better quantitative assessment.

The concave pattern develops when lung compartments have widely differing expiratory time constants causing regional inhomogeneity (83) as is certainly the case in obstructive lung disease with peripheral increase in airway resistances, with the slowest emptying compartments contributing disproportionally to flows near residual volume, resulting in a curve with the familiar exaggerated 'tail'. It is not surprising, therefore, that even though the underlying mechanics determining FEV_1 and the concave pattern overlap, they are not necessarily equivalently strong physiological signals at different disease stages. They may however be quite complementary, not only in assessing airflow obstruction overall but especially in detecting early obstructive small airway disease (84).

It seems reasonable that to detect and assess early disease we need a method that is sensitive to inhomogeneous airway emptying because this almost certainly precedes the development of the more advanced obstruction for which we use currently the standard FER. Highly sophisticated technology is currently being developed to measure this inhomogeneous lung emptying, but it could well be that much of this information is already available in the expiratory flow-volume curve if only it can be harnessed.

Strong evidence that a concavity confined in the terminal portion of the curve is most likely to be associated with

small airways dysfunction came from further studies that compared flow-volume curves obtained breathing gases of widely differing gas density which showed that maximal flows near the terminal portion of the flow-volume curve predominantly reflect small airway function (85). This is also consistent with studies using wave speed mechanics (86) and the equal pressure point theory (72) which predicts that the flow-limiting segment developed during forced expiration moves peripherally into progressively smaller airways as lung volume falls and especially when peripheral airway resistance increases.

The clinical value of quantifying concavity has been under-appreciated although demonstrated spirometrically in different populations (84,87-92). The study by Kraan *et al.* (89) was of particular interest as it provided strong evidence that although the reduction in FEV_1 and the degree of concavity are related, they do not necessarily measure the same things; for example they were differentially affected by bronchodilator and anti-inflammatory treatment. Schachter *et al.* (87) showed that although cotton workers had abnormal spirometry, the concave pattern was only present in current cigarette smokers. Another study showed the degree of concavity was greater in those with a smoking history and people with breathlessness and wheezes (88).

New indices to quantify concavity

Visual assessment of concavity in the flow-volume curve is highly subjective and cannot reliably be used to assess an abnormal degree of concavity. What is needed is a practical and easily understood numerical index to quantify concavity with clearly defined limits of normal. Although a number of methods have been described (83,87,93) they are complex or difficult to apply routinely and none has been incorporated into commercial spirometry software or clinical guidelines.

However, we describe two indices for estimating concavity (global and peripheral) with preliminary data comparing these with conventional spirometric variables, in a randomly selected population of adults aged >40 years in Australia. The *global index* is based on $FEF_{50\%}$ and quantifies concavity that usually involves the entire descending limb, whilst the *peripheral index* is based on the $FEF_{75\%}$ and independently quantifies concavity present near the terminal portion of the curve. The degree of concavity is obtained by calculating the percentage decrease of the measured flows from the corresponding idealised reference flows (*Figure 1*).
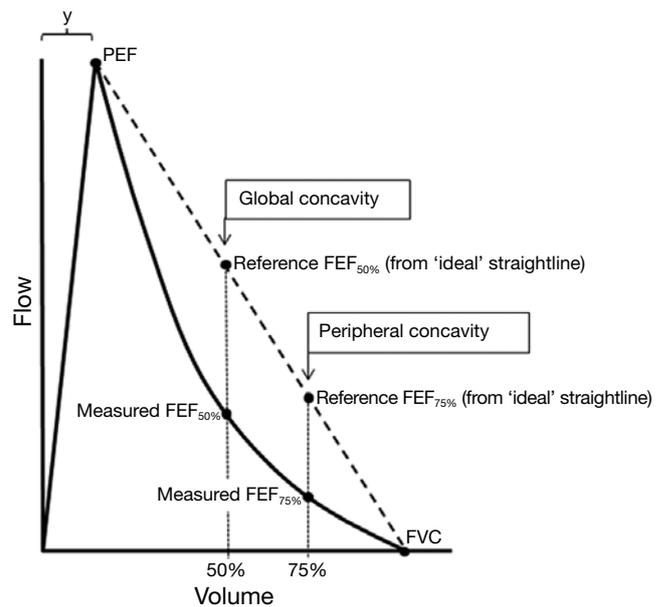
Figure 1 Variables used to quantify global and peripheral concavity (see text). Measured $FEF_{50\%}$ and measured $FEF_{75\%}$ are the forced expired flows when 50% and 75% of the FVC has been expired. Reference $FEF_{50\%}$ and Reference $FEF_{75\%}$ are the reference flows that would be obtained if the flow-volume curve had zero curvature i.e., a linear descending limb (dotted line). The variable, y, is the volume to peak expiratory flow (PEF); a value of 0.6 L can be assumed for this. In this example, global concavity is approximately 50 Units and peripheral concavity is approximately 65 Units.

Global Concavity $=100*$ (reference $FEF_{50\%}$—measured $FEF_{50\%}$)/reference $FEF_{50\%}$

Peripheral Concavity $=100*$ (reference $FEF_{75\%}$—measured $FEF_{75\%}$)/reference $FEF_{75\%}$

The measured $FEF_{50\%}$ and measured $FEF_{75\%}$ are obtained from the subject's flow-volume curve. The two corresponding reference flows are calculated assuming that the descending limb is a straight line from PEF to end-expiration (*Figure 1*) and therefore has no curvature:

Reference $FEF_{50\%}$ = PEF*(FVC/2)/(FVC-y)

Reference $FEF_{75\%}$ = PEF*(FVC/4)/(FVC-y)

The variable, y, is the volume expired to PEF (*Figure 1*) and although ideally should be measured from the curve, assuming a fixed value of 0.6 litres leads to little error. The calculated indices are dimensionless with units ranging from zero (no concavity) to a theoretical limiting value

Table 1 Spirometry and concavity data mean and standard deviation (SD) from the Tasmanian BOLD population (17)

	Males (n=164); mean (SD)		Females (n=223); mean (SD)	
	Never smoked (n=66)	Ever smoked (n=98)	Never smoked (n=115)	Ever smoked (n=108)
Post-BD spirometry				
FEV_1, % predicted	101.5 (13.2)	93.3 (15.5)	98.8 (16.6)	99.0 (16.3)
FVC, % predicted	101.3 (12.4)	97.5 (14.1)	99.5 (15.2)	99.4 (13.2)
FER, absolute %	76.0 (6.8)	72.5 (9.3)	77.2 (8.0)	73.8 (9.5)
Acute reversibility				
FEV_1, % change	4.4 (4.1)	3.7 (6.6)	3.2 (7.0)	3.6 (5.8)
FER, absolute change	2.3 (4.2)	2.8 (4.5)	2.4 (4.2)	2.2 (4.5)
Global concavity units				
Pre-BD	32.7 (21.6)	40.4 (22.3)	22.9 (24.2)	32.0 (26.9)
Post-BD	27.5 (22.6)	35.2 (23.6)	13.4 (26.4)	25.6 (29.4)
Peripheral concavity units				
Pre-BD	67.1 (19.0)	74.0 (14.0)	63.9 (19.4)	69.5 (18.5)
Post-BD	59.1 (24.1)	68.3 (18.0)	56.9 (21.6)	63.3 (21.4)

BD, bronchodilator; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; FER, forced expiratory ratio.

approaching 100 (maximum concavity). Negative values are possible and indicate that that the curvature of the descending limb is convex (no concavity). These indices (Concavity Units) are easily incorporated into spirometry software, are independent of the size of the flow-volume curve and closely mirror the intuitive way many clinicians visually assess the degree of concavity, mentally adding the straight line, but with more objectivity.

Our exploratory analysis of global and peripheral concavity involved 387 (223 females, 164 males) randomly selected subjects from Tasmania who had participated in the BOLD Australia study (17). Spirometry and the degree of concavity were obtained from baseline and post-bronchodilator flow-volume curves measured using the Easyone ultrasonic spirometer (ndd Medizintechnik) that met ATS/ERS acceptability and repeatability criteria (75). The age range of subjects was 42-87 years, with mean age 59.4 years for males and 58.3 for females. A higher proportion of males had ever smoked (60%) compared with females (48%) and males had substantially higher lifetime tobacco consumption (median 24 versus 11 pack years). Overall subjects who had ever smoked, especially males with the highest lifetime tobacco consumption, had a greater degree of global and peripheral concavity compared with never smokers (*Table 1*). The degree of global and peripheral concavity decreased after the administration of a bronchodilator, in both the male and female subjects who

had ever smoked or had never smoked. Of note, even in people who had never smoked the presence of both patterns of concavity was a common finding in this older population.

The limits of normal for concavity were estimated separately for males and females using post-BD data from subjects who had never smoked, with FER >0.7 and reversibility of FEV_1 <10%. Thus, an abnormal degree of concavity was defined as present in males if global concavity >34.8 Units or peripheral concavity >61.2 Units, and in females if global concavity >26.3 Units and peripheral concavity >63.1 Units. The LLN for $FEF_{25-75\%}$ was based on reference values from Hankinson *et al.* (25).

In this Tasmanian population, the prevalence of abnormal global and peripheral concavity was far higher than estimated based on either GOLD criteria or $FEF_{25-75\%}$ (*Table 2*). It is of interest that the presence of an abnormal degree of concavity confined solely to the terminal portion of the curve (global > ULN plus peripheral < ULN) was not uncommon. This pattern was present in 73 (19%) of participants overall of whom only four had abnormal FER (<0.7).

Both the FER and degree of concavity are independent of the size of the flow-volume curve. *Figure 2* shows that there is a strong non-linear relationship between FER and our measures of concavity. The horizontal and vertical lines are the limits of normal for FER (GOLD) and concavity, respectively. According to clinical guidelines, subjects falling to the right of the vertical line have a normal FER

Table 2 Comparison of prevalence rates of abnormal conventional spirometry indices and abnormal concavity for the Tasmanian BOLD population (17)

Index	Criterion for abnormal	n, prevalence (%)	
		Males (n=164)	Females (n=223)
FER (± FEV_1 % pred.)	FER <0.7 (GOLD any stage)	46 (28.0)	47 (21.1)
	FER <0.7 + FEV_1 ≥80% pred. (GOLD stage I)	31 (18.9)	27 (12.1)
	FER <0.7 + FEV_1 ≥50% to <80% pred. (GOLD stage II)	14 (8.5)	17 (7.6)
$FEF_{25-75\%}$	$FEF_{25-75\%}$ < LLN (25)	11 (6.7)	20 (9.0)
Global Concavity Units	> ULN	76 (46.3)	89 (39.9)
Peripheral Concavity Units	> ULN	108 (65.9)	110 (49.3)
Pure peripheral Concavity	Global < ULN + peripheral > ULN	37 (22.6)	36 (16.1)
GOLD, global initiative for chronic obstructive lung disease (15).			
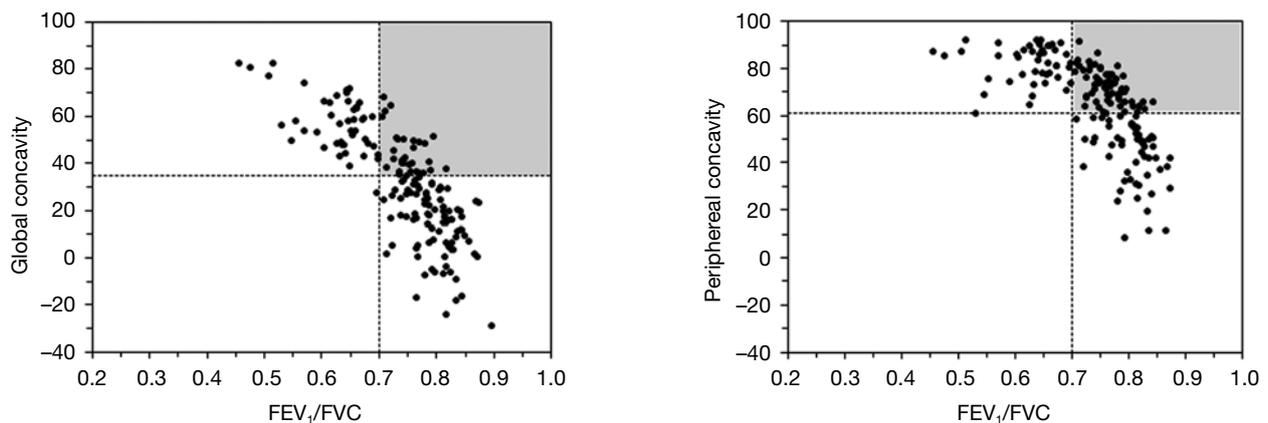
Figure 2 Post-bronchodilator forced expiratory ratio (FER) of FEV_1/FVC plotted against global and peripheral concavity in male participants. The horizontal and vertical lines are the limits of normal for FER (15) and concavity, respectively. The shaded quadrant identifies subjects with normal FER but an abnormal degree of concavity (see text).

(no airflow obstruction) and from our data those above the horizontal line have an abnormal degree of concavity. The upper right quadrant (shaded area in *Figure 2*) is of special interest because it identifies subjects without airflow obstruction defined by the FER but who have an abnormal degree of concavity. This may be useful in detecting airflow obstruction that is unseen by conventional analysis of spirometric data. This requires further investigation as does the relationship between concavity and symptom scores, and whether the association is stronger than between symptoms and FEV_1. The ability to fully utilize the large amount of information obtained in modern spirometry could have great potential, opening a way to introduce the insights about early small airway dysfunction from classical physiology into the clinic without a need for additional complex equipment.

Conclusions

The standard respiratory function test for case detection of COPD is spirometry, with the criterion for diagnosis defined in guidelines being based on FER and the severity being based on FEV_1. However, using this approach is poor at detecting early disease in the small airways. Improved, although more complex, tests are being developed to recognise such early cases but we have shown that by using all the information available from the spirometric expiratory flow-volume curve, and especially by quantifying the degree of concavity, that this may be in itself more sensitive and specific for small airways disease. However, even the current means of diagnosing relatively more severe disease that is detectable by the FER threshold is poorly taken up in primary care, despite the benefits that could be

achieved with smoking cessation and pharmacological and non-pharmacological interventions to improve patients' well-being. The reasons are not completely understood but include attitudes of both doctors and patients to COPD. The potential importance of detecting early fixed airway obstruction for prevention of lung cancer and non-respiratory end-organ disease also needs to be better highlighted in public health campaigns.

Acknowledgements

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Implementing clinical guidelines for chronic obstructive pulmonary disease: barriers and solutions

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Abstract: Chronic obstructive pulmonary disease (COPD) is a complex chronic lung disease characterised by progressive fixed airflow limitation and acute exacerbations that frequently require hospitalisation. Evidence-based clinical guidelines for the diagnosis and management of COPD are now widely available. However, the uptake of these COPD guidelines in clinical practice is highly variable, as is the case for many other chronic disease guidelines. Studies have identified many barriers to implementation of COPD and other guidelines, including factors such as lack of familiarity with guidelines amongst clinicians and inadequate implementation programs. Several methods for enhancing adherence to clinical practice guidelines have been evaluated, including distribution methods, professional education sessions, electronic health records (EHR), point of care reminders and computer decision support systems (CDSS). Results of these studies are mixed to date, and the most effective ways to implement clinical practice guidelines remain unclear. Given the significant resources dedicated to evidence-based medicine, effective dissemination and implementation of best practice at the patient level is an important final step in the process of guideline development. Future efforts should focus on identifying optimal methods for translating the evidence into everyday clinical practice to ensure that patients receive the best care.

Keywords: Pulmonary disease; chronic obstructive; clinical practice guidelines; health services; evidence-based practice

Introduction

Chronic obstructive pulmonary disease (COPD) is a complex lung disease that is characterised by progressive worsening of airflow limitation, punctuated with acute exacerbations that if severe, frequently require hospitalisation (1). COPD is the third leading cause of mortality worldwide (2). Several evidence-based guidelines for the diagnosis and management of COPD are available to clinicians, including the GOLD guidelines internationally and the COPD-X Plan: Australian and New Zealand Guidelines for the management of COPD. Although they are widely available, the knowledge and implementation of these guidelines are highly variable in actual clinical practice. Many barriers to the implementation of management guidelines by clinicians have been identified (3), but relatively few studies have investigated methods for enhancing the use of guidelines. This review explores these critical issues, gaining insight from efforts in clinical guidelines for other chronic diseases, and applying these principles to improving uptake of the COPD guidelines amongst clinicians.

Benefits of adhering to clinical recommendations contained in guidelines for COPD

The goals of therapy in COPD are to reduce symptoms

and improve quality of life, and reduce future risk of adverse outcomes including exacerbations, hospitalisations and mortality. The evidence base for achieving these goals are encapsulated in international (1) and national clinical guidelines available throughout the world. By following these guidelines, clinicians will ensure that they are translating the best available evidence into their everyday clinical practice.

Spirometry is the gold standard diagnostic test to confirm fixed airflow limitation in individuals with dyspnoea, chronic cough or sputum production, and risk factors for COPD (1). Spirometric diagnosis of COPD at any stage is an essential step to ensure an accurate diagnosis and to guide therapy (4). The benefits from using non-pharmacological therapies in COPD are substantial—smoking cessation reduces lung function decline (5), influenza and pneumococcal vaccination decrease the risks of these infections (6,7), and pulmonary rehabilitation improves quality of life, increases exercise tolerance and reduces frequency of hospitalisations (8,9). In addition, pharmacological therapies provide benefit for patients, with long-acting bronchodilators (10-15) and inhaled corticosteroids (16) acting to reduce dyspnoea, improve quality of life and lung function, decrease risk of exacerbations and possibly reduce mortality (17). Early recognition and appropriate treatment of acute exacerbations with bronchodilators, systemic corticosteroids and antibiotics, where appropriate, reduce symptoms, shorten time to recovery and reduce risk of relapse (18,19).

Preparation of evidence-based clinical guidelines: the Australian COPD-X guidelines as an example

Many national guidelines have been written and adapted for use in specific countries. In the process of evidence-based guideline development, it is particularly important that the developers regularly review and critically appraise the evidence, to ensure that the guidelines are up-to-date, high quality, clinically relevant and beneficial to healthcare professionals and patients (20). As an example, many of us have been involved in the Australian COPD-X clinical guidelines developed by Lung Foundation Australia. COPD-X stands for Case detection and Confirm diagnosis, Optimise function, Prevent deterioration, Develop management plan of care, and manage eXacerbations. These guidelines were initially published in 2003 in the *Medical Journal of Australia* (21) and an update was published in 2006 (22). Since then, regular updates have been available on a dedicated website administered by the Lung Foundation (www.copdx.org.au), with the latest

version (2.37) recently published online.

The guidelines are prepared by the Lung Foundation Australia's COPD Guidelines Committee, which consists of eight clinical members plus executive administrative support. Conflict of interest statements from each Committee member are recorded on an annual basis and published on the COPD-X website. The Committee meets quarterly to appraise the latest published evidence and make recommendations on updating the guidelines. Prior to each meeting, searches are carried out in the Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects and PubMed, using detailed search strategies (available on request) which include COPD and emphysema as key words.

At each meeting, search results are discussed to decide whether full papers should be obtained for review. At the subsequent meeting, committee members discuss their appraisal of the papers reviewed and recommend whether the papers should be cited in the guidelines, with any additional form of wording included. After an approval process, Lung Foundation Australia publishes online updates of COPD-X (typically two updates per year) to include the changes agreed at recent meetings of the Committee. An email is sent to all the registered users of the COPD-X website informing them when an update has been made and what changes have occurred. A COPD-X Concise Guide for Primary Care, based on the full guidelines, will be released by Lung Foundation Australia in November 2014 (www.copdx.org.au).

Low concordance with guideline recommendations

A major issue is low awareness of clinical guidelines, which may subsequently translate into low adherence to guideline recommendations, and potentially suboptimal clinical care for patients in primary, secondary and tertiary care. Even with detailed processes for preparation and implementation of guidelines, such as the Australian COPD-X guidelines above, observational studies suggest that clinician knowledge of management guidelines is relatively low world-wide, both for chronic diseases generally and specifically for COPD.

Chronic disease guidelines

Many lessons can be learned from use of clinical guidelines in chronic diseases other than COPD, and applied in principle to COPD guidelines. As examples, comparison of

studies examining chronic disease management reveals that many cardiovascular-related guidelines are largely adhered to; however, lower adherence has been generally observed to guidelines for metabolic diseases such as diabetes and osteoporosis (23-28).

Cardiovascular disease

In general, good adherence has been observed for cardiovascular disease guidelines (23,24). For hypertension, a study of 410 physicians in Finland found an 89% adherence rate to the guidelines, and that implementation improved in larger centres with more structured health care systems (24). Another study of general practitioners (GPs) in Austria found 83% adherence to cardiovascular disease and diabetes guidelines (23). However, when non-adherence occurred, factors included lack of familiarity with guidelines, even after education, and disagreement with guideline treatment recommendations (23,26,28,29).

Metabolic diseases

In general, rates of adherence to guidelines in the management of metabolic diseases have been observed to be relatively low. Studies of adherence to guidelines for screening of gestational diabetes found very low rates of adherence in obstetric units in France (25) and Canada (26). Factors identified included lack of familiarity with new guidelines, unfamiliar screening measures, poor acceptance and feasibility, difficulty with administration of recommendations, patient non-adherence, and ambiguity in guideline recommendations (25,26). Additionally, a retrospective survey of 200 GP referral letters to a hospital in Scotland showed that the introduction of local type 2 diabetes guidelines had no significant effect on screening for patient complications, or on GP referral letter content about complications of diabetes (27). In a survey of Canadian GPs, 35% of respondents had not read or been aware of the latest osteoporosis guidelines (28).

Though many guidelines for chronic diseases have been carefully developed, relatively low adherence to guideline recommendations—particularly for metabolic disease but less so for cardiovascular diseases—may result in suboptimal healthcare. Many barriers to adherence with chronic disease guidelines have been identified, as outlined for the examples above. Hence to improve adherence to guidelines, research has focused on strategies to target health professional education, decision-making algorithms (including electronic systems), and reminders at the time of consultation, and continuous quality assurance programmes.

COPD guidelines

Correct diagnosis and subsequent appropriate treatment selection have been the most common areas of deficiency identified in adherence to COPD guidelines. Most studies have focused on outpatient management of COPD, particularly primary care. A cross-sectional study of 455 primary care physicians and 243 physicians practicing in a hospital system, published in 2004, found that only 55% of Swiss physicians used spirometry in the diagnosis of COPD, and only one-third knew the GOLD criteria for COPD (30). Knowledge of the indications for use of inhaled steroids and referral to pulmonary rehabilitation were also low. A recent cross-sectional study of 593 GPs in Shanghai found that whilst 55% of GPs recognized the different severity classifications, only 8% of patients with COPD received prescriptions in accordance with GOLD guidelines (31). Thus, although COPD guidelines are widely available and accessible in print and online, studies suggest that clinician awareness of their specific recommendations is generally low, despite many efforts for their implementation and dissemination.

Several studies in COPD have demonstrated that actual clinical practice may deviate significantly from guideline recommendations (*Table 1*). A retrospective study of 450 outpatients with stable COPD in the US (33) found that 56% of patients received guideline-concordant pharmacotherapy, based on the 2007 GOLD guidelines available at that time (35). There was a significant relationship between suboptimal treatment and adverse outcomes: patients who received guideline-discordant treatment had nearly twice the number of exacerbations as those who received guideline-concordant care. A cross-sectional study of 1,517 primary care patients with COPD in the US found that 27% of patients had spirometry documented within the previous year, 25% had comorbid conditions appropriately managed, and 32% had appropriate measures in place for risk reduction (34). In a Swiss study, GP prescription of long-acting bronchodilators or inhaled steroids was guideline-concordant in only 20% of patients with GOLD spirometry stage I and II COPD, whereas 64% of patients with more severe stage III and IV COPD received guideline-concordant treatment (32). Finally, a retrospective study of 1,185 patients with moderate to severe COPD in Slovenia found that those who died during follow-up were more often found to have experienced suboptimal management according to guidelines when compared to patients who were alive at the end of the

Table 1 Examples of studies of actual clinical practice compared to COPD guidelines

Study	Methods	Outcomes
Jochmann, 2010 (32)	Prospective study of 139 GPs and 615 patients with COPD. GPs completed survey of management of each patient. The authors compared actual management with recommended management based on disease severity	➢ 44% of patients did not fulfill GOLD diagnostic criteria for COPD ➢ Only 5% of patients received pulmonary rehabilitation ➢ 36% of all patients with COPD were prescribed a minimum of a short acting bronchodilator ➢ Prescription of long-acting bronchodilators or inhaled corticosteroids was guideline-concordant in 20% of patients with GOLD stage I or II disease and 64% of patients with stage III or IV disease ➢ 6% of patients with stable disease received inappropriate systemic steroids
Sharif, 2013 (33)	Retrospective study of the management of 450 patients with COPD treated in an academic medical centre in the USA. Actual treatment was compared with indicated treatment based on GOLD guidelines	➢ 56% of patients received treatment concordant with GOLD guidelines ➢ Patients co-managed by a primary care physician and respiratory specialist were most likely to receive guideline-concordant treatment ➢ Patients who received guideline-discordant treatment had nearly twice the number of exacerbations as those who received guideline-concordant care in the year following spirometry
Belletti, 2013 (34)	Cross-sectional study of management of 1,517 patients with COPD in US primary care centres with retrospective chart review of medical records	➢ 27% of patients underwent spirometry in previous year ➢ 25% were having comorbid conditions appropriately managed ➢ 32% had appropriate measures in place for risk reduction ➢ 3% of patients met all three guidelines components for (I) spirometry; (II) management of comorbid conditions; and (III) risk reduction measures

GP, general practitioner.

follow up period (including less long-acting inhaled medicine prescribed by their medical practitioners) (36). Overall, these findings support the value of guideline-based care in improving patient outcomes in COPD, and clearly demonstrate areas of practice that can be enhanced.

Barriers to guideline adherence

Specific barriers to adherence to COPD guidelines have been identified in observational studies. In one study of 154 general internists in the US, factors associated with reduced concordance with clinical guideline recommendations included low familiarity with COPD guidelines, perceived low self-efficacy for use of guidelines, and time constraints (37). In another US study, only 32% of 500 primary care physicians reported high familiarity with the GOLD COPD guidelines (38). In comparison, a larger proportion (76%) of the same group of physicians rated themselves as very familiar with guidelines for management of hypertension, indicating a relative disparity in awareness of guidelines for these two common chronic conditions. A qualitative Australian study, using semi-structured interviews of nine hospital-based registrars or interns, and seven GPs, found that barriers to implementation of evidence-based

recommendations for COPD included lack of supportive enablers and complexity of the behavioural change needed in their patients (39).

Approaches to improving adherence to guidelines

Improving adherence to chronic disease guidelines

A number of methods of improving guideline implementation have been investigated in chronic diseases other than COPD that could feasibly be applied to COPD guidelines.

Distribution of guidelines

Having knowledge itself does not necessarily translate to clinical practice; therefore implementation and distribution strategies must be utilised. Use of brief summaries or storyboards of guidelines posted in clinical areas are associated with a positive impact on provider knowledge (40). Other distribution methods include mailing guidelines directly to clinicians or generating publicity prior to guideline publication (41). The act of voluntarily downloading guidelines also demonstrated active participation and greater

likelihood that the guidelines would be considered useful.

Clinician professional development

Continuing medical development is another commonly used method with the aim to increase clinician adherence to guidelines. A study of infection management found a significant improvement in compliance with infection control protocols after active education when compared to no intervention (42). A third arm, the passive intervention group where education material was offered but not actively made available, resulted in only 34% of participants engaging in education, compared to the 91% in the active group. However, studies of diabetes care and lower back pain management showed no significant difference in guideline adherence after an educational program (43,44). A Cochrane review of 81 studies found that continuing medical development meetings can lead to a small improvement in practice and patient outcomes (45). Factors associated with effective educational meetings were higher attendance rates and a mix of didactic and interactive sessions.

Electronic health records (EHR)

EHR usage has been associated with improved provider knowledge (40). However, in a 2-year study comparing the EHR to non-EHR primary care practices, there was no significant difference in adherence to diabetes management guidelines between these two groups. In other studies of diabetes management before and after implementation of EHRs, EHRs have been demonstrated to improve attainment of healthcare management standards. For example, EHR implementation increased rates of patients with diabetes having their glycated haemoglobin (HbA$_{1c}$) level checked at the appropriate intervals, being prescribed antihypertensive medication when indicated, and receiving pneumococcal vaccination (46). EHRs have also been shown to improve treatment intensification following abnormal HbA$_{1c}$ levels (47), and reduce both emergency department (ED) visits and hospitalisations in patients with diabetes (48).

The cost efficiency of EHRs is still controversial, and there are many factors to consider regarding their use. Ultimately, EHRs will be most successful when they can meet the needs of physicians and their patients, promote quality care, and maximise efficiency (49).

Reminders about guideline recommendations

Having guidelines in a readily accessible manner, when they are required at point-of-care, has been shown to improve guideline adherence (50). Similarly, establishing checkpoints, deadlines or cues during a consultation has been associated with improved provider knowledge (40). This has been found to be effective in increasing guideline adherence, which could then be translated into significantly improved patient quality of life (51,52). Reminders have been associated with positive clinician behavioural change, since relevant information is being presented during the clinical decision-making process (28,53). A Cochrane review of 32 studies found that computer-generated reminders for tasks such as ordering screening tests or giving vaccinations provided to clinicians on paper resulted in a median 11% improvement in process of care measures compared to usual care (54).

Though the reminder system has proven to be effective, it has been suggested that physician perception of the benefit of the reminder may also influence their usage. One study has shown that prompts perceived to be regulatory and for enforcement will be used less than prompts perceived to be for guidance (55). A Cochrane review of audit and feedback systems for clinicians found that the success of such programs depends on several factors (56). Characteristics associated with behavioural change in response to these programs include poor baseline performance, feedback from colleagues, repeated feedback, multimodal communication of feedback, and tangible goals with a clear process to achieve them.

Decision support systems

CDSS are software programs that can assist clinician decision-making. One randomised study published in 1999 utilising a CDSS on hypertension management showed no clinically significant improvement (57), possibly attributed to less than expected use of the CDSS. In contrast, a Cochrane review of 42 trials found that point-of-care computerised advice for drug dosing improved objective parameters for several drugs, including anticoagulants, insulin, and antibiotics, although this did not translate to improvements in mortality (58).

Multifaceted health care systems

Evidence is mixed on whether combining implementation techniques have a synergistic effect on adherence to guidelines. A multifaceted approach to gestational diabetes, using pamphlets, reminders and meetings, showed significantly increased guideline adherence in the prescription of screening tests after 2 years of implementation (25). A study of cervical cancer screening showed significant improvements in most of the clinical

Table 2 Examples of studies of methods to improve adherence to clinical guidelines for COPD management

Study	Methods	Outcomes
Ulrik, 2010 (63)	Cross-sectional surveys of 124 GPs in Denmark at baseline and 12 months after completion of an educational program. The management of 1,716 and 1,342 patients with COPD was assessed in the first and second surveys, respectively. The educational program consisted of individual meetings with specialists, expert symposia, individual review of audit data, and included GPs and their staff	➢ Significant improvements were observed in recording of disease severity, smoking status, BMI, dyspnoea severity, and FEV_1/FVC ratio ➢ Significant increases were observed in smoking cessation counseling, teaching of correct inhaler technique, promoting exercise, and pulmonary rehabilitation referrals ➢ Inappropriate prescription of inhaled corticosteroids in patients with mild disease decreased (pre-education 76% *vs.* post-education 45%)
Bertella, 2013 (64)	Retrospective review of 12 GPs in Italy caring for 328 patients with COPD at baseline and 12 months after completion of an educational program for GPs. Educational program involved lectures, spirometry training, and specialty phone consultation service	➢ The educational program did not significantly affect use of spirometry or chest X-rays ➢ A small increase in recording of smoking status was observed with the educational program

recommendations, with use of software modules which facilitated selection, attendance monitoring, follow up and also provided reminders (59). A study conducted of beta-blocker usage in patients with heart failure observed little improvement in prescription after using provider education alone and provider/patient notification, but significant improvements with the use of a nurse facilitator (60). However in diabetes management, implementation methods such as chart audits, performance feedback, reminders and computerized supports improved only 4 of 9 criteria in the first year of use, with effectiveness falling to 2 of the 9 criteria after the second year of implementation (61).

In summary for chronic disease guidelines, numerous methods for improving the implementation of guidelines have been studied, however the results are mixed. Professional education, EHRs and multifaceted implementation programs have shown benefit in some studies, but not in others. Programs that distribute guidelines in a way that promotes active participation by clinicians are more likely to have a positive effect, and interactive reminder systems at the point of care provide an effective solution for guidelines when they are most needed. Further work is needed to identify the aspects of these implementation programs that are beneficial.

Improving adherence to COPD guidelines

There is increasing recognition of the importance of independent research to further clarify the most effective methods for developing and implementing evidence-

based guidelines (20). The publication of clinical practice guidelines is only the first step in a process that ends with an actual change in clinician behaviour. The importance of effective guideline dissemination methods cannot be overlooked. Grimshaw *et al.* (62) reviewed the studies of guideline dissemination methods and the role of guideline developers in this process. They concluded that developers must take an active role in including methods for disseminating and implementing their guidelines.

Most research to date on enhancing uptake of COPD guidelines has focused on improving clinician knowledge and awareness of guidelines through education sessions (*Table 2*). Studies have shown mixed results, with some suggesting minor improvements in management following the intervention, and others showing no effect.

Two observational studies have evaluated the effect of an education program for GPs on COPD guideline uptake. A study of GPs in Denmark found that several guideline-related indices of diagnosis and management improved 12 months after a comprehensive education program (63). Better use of spirometry to classify disease severity and a reduction in inappropriate use of inhaled corticosteroids were observed. In contrast, a small study of GPs in Italy found no significant change in management following an educational program that consisted of lectures, spirometry training, and specialty phone consultation service (64). The discrepancy between these study results may be due to pre-existing levels of guideline uptake, contrasting styles of educational programs, different health system contexts or

inadequate sample size.

Point-of care checklists may also be useful in hospital clinical practice. Previously, members of our group have undertaken a prospective pilot study of the use of an inpatient checklist to promote evidence-based recommendations (unpublished data, presented at the Thoracic Society of Australia and New Zealand Annual Scientific Meeting 2012) (65). Focus groups, statewide stakeholder consultation and a literature review were used to develop a paper-based, one page Inpatient Checklist for acute exacerbations of COPD (AECOPD), based on the Australian COPD-X guidelines. Demographic, process of care and patient outcome data for AECOPD admissions were collected in pre-checklist [2010] and checklist-implementation [2011] phases at one tertiary referral hospital. Two groups of admissions were studied in pre-checklist (n=42) and checklist-implementation (n=68) groups. Adherence to checklist use by ward medical staff in a respiratory ward was 51% (35 of the 68 checklist-implementation admissions).

Concordance with COPD-X Plan recommendations was high overall for patient assessment (e.g., admission chest X-ray 100%) and initial treatment (e.g., inhaled bronchodilators 100%, systemic corticosteroids 79%, and antibiotic therapy 91%). Concordance was lower for inpatient consideration of longer-term issues such as referral to pulmonary rehabilitation (36%). Checklist use was associated with significantly increased rates of arterial blood gas analysis (86% *vs.* 61%, P=0.02), sputum microbiology testing (83% *vs.* 58%, P=0.02) and influenza vaccination assessment (71% *vs.* 51%, P=0.04), compared with admissions without checklist use. Median length of stay did not change significantly with checklist use. In this pilot study, checklist use was associated with increased rates of several recommended strategies for management of inpatients with an AECOPD. This study has shown that there is opportunity to improve aspects of inpatient care for COPD and to refine methods of guideline dissemination (65). There may be even better uptake with electronic checklists for COPD, which could be used in both inpatient and outpatient settings to improve guideline adherence.

Lessons from implementation of COPD, asthma and pneumonia guidelines

While the individual interventions recommended by clinical practice guidelines are evidence-based, there are relatively few studies assessing patient outcomes following the implementation of COPD guidelines per se (66). Most studies of guideline implementation to date have focused on healthcare process rather than patient outcome measures. One small randomised controlled trial assessed change in mean peak expiratory flow rate (PEFR) in patients in general practice patients following a comprehensive guideline implementation program (67). Patients in the intervention group experienced a statistically significant improvement in mean PEFR, as well as respiratory symptoms and pain scores. However this improvement was small when compared to the control group receiving usual care. A large RCT of primary care practices in the US has been planned, which will incorporate systems such as computerised patient activation tools and web-based COPD guidelines to improve guideline uptake (68).

Beyond COPD, there are several examples of guideline implementation leading to improved patient outcomes, in particular in the treatment of pneumonia and asthma. A US study found that implementing pneumonia treatment guidelines significantly reduced 30-day mortality of elderly patients (69). The authors hypothesised that the improved patient outcomes were due to earlier and more accurate identification of high-risk cases, with a significant improvement in utilisation of appropriate antibiotics during the same time period.

In Australia, a multi-faceted approach to improving awareness and management of asthma was implemented with success in the early 1990s, in the form of new clinical guidelines published as an initiative of the National Asthma Council (originally the National Asthma Campaign). These guidelines were published in the Asthma Management Handbook, and now the Australian Asthma Handbook (70). The campaign involved promoting the best practice management for asthma to healthcare professionals, especially in primary care, and the general public (71). This highly effective approach likely played a role in improved asthma management and reduced asthma mortality (by 70% since the 1980s) after implementation (72,73).

Conclusions and future studies

COPD is a major cause of morbidity and mortality worldwide. Guidelines for the diagnosis and treatment of COPD are widely available, however, concordance with these guidelines remains sub-optimal. Numerous barriers to guideline adherence have been identified, but studies assessing methods for improving the uptake of guidelines in COPD management are limited to small-scale non-randomised studies. Given the significant resources

invested in guideline development and the cost of management of COPD in general, there is a need for better strategies to ensure effective guideline implementation and optimise their use. Future studies should evaluate implementation strategies used in the management of other diseases for COPD. These studies could assess a combination of techniques to ascertain whether a synergistic response exists and to find the most efficient model with greatest marginal benefit for each different disease. This could allow the development of an optimal protocol that can assist with implementation of guidelines. There are many interventions that have a proven mortality or symptom benefit for patients with COPD. Given the benefits of these treatments, it would be logical to assume that guidelines comprising these interventions would lead to better patient outcomes. There are, however, very few studies assessing the effect of COPD guideline implementation on patient outcomes, and this is an area that requires further research. Clinical guidelines are an important modality for communicating evidence-based recommendations to clinicians at the point of care. The development of guidelines is an important early step, but more research is needed to determine the most effective ways to translate the evidence into everyday clinical practice.

Acknowledgements

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JTD special edition 'Hot Topics in COPD'—The microbiome in COPD

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Abstract: The pathogenesis of chronic obstructive pulmonary disease (COPD) and its exacerbations, are intricately linked to colonisation and infection with bacteria and other microbes. Despite their undeniable importance, we have a poor understanding of the complex relationships between COPD phenotypes, physiology, cellular and molecular biology and the roles of colonising microbe or infecting pathogens. The management algorithms for the care of patients with COPD that include microbial influences, have almost exclusively been developed using microbial methods that were entirely dependent on the ability to grow bacteria on suitable media. The shortcomings of this approach are becoming clear now that it is possible to completely and accurately define the microbial ecology of ecosystems using genomic methods, which do not rely on the ability to cultivate the organisms present. Whilst our appreciation of the relationships between some bacterial ecosystems and the organ in which they reside in humans is now relatively advanced, this is not true for lung. This perspective serves to highlight the growing importance of including an accurate description of bacterial ecology in any attempt to decipher the pathobiology of COPD. While this field is in its infancy, there is significant potential to gain new insights which will translate into more rational and effective treatment algorithms for patients with COPD.

Keywords: Chronic obstructive pulmonary disease (COPD); microbiome; metagenomics

Introduction

Throughout history many inventions have radically altered the way we are able to perceive the world—the telescope, the microscope, lenses for those with refractive errors and cochlear implants for those with deafness. A similar revolution has recently occurred in the way we are able to observe the microbial world. The genomic methods and biostatical approaches which can now be employed to comprehensively determine the structure of microbial communities have transformed the study of microbial ecology and will have a similar impact on our understanding of human health. What should be self-evident—that we have evolved not in a sterile world, but in one teeming with microbes (our cells are outnumbered 10:1 by our gut bacteria alone) where each species has their own

evolutionary agenda, but is also open to collaboration—has been brought into clear focus. The idea that infectious disease is a two-sided battle to the death has been exposed as simplistic, with symbiotic relationships between host and microbe being at least as prevalent and important to human health in some organs (especially the gut, but possibly also the lung). The implications for diseases like COPD where microbial colonisation and infection is central to pathogenesis are obvious and are the subject of this perspective.

What is the microbiome and how can we 'see' it?

The microbiome can be defined as the collective sum of microorganisms (and their genomes) inhabiting a given
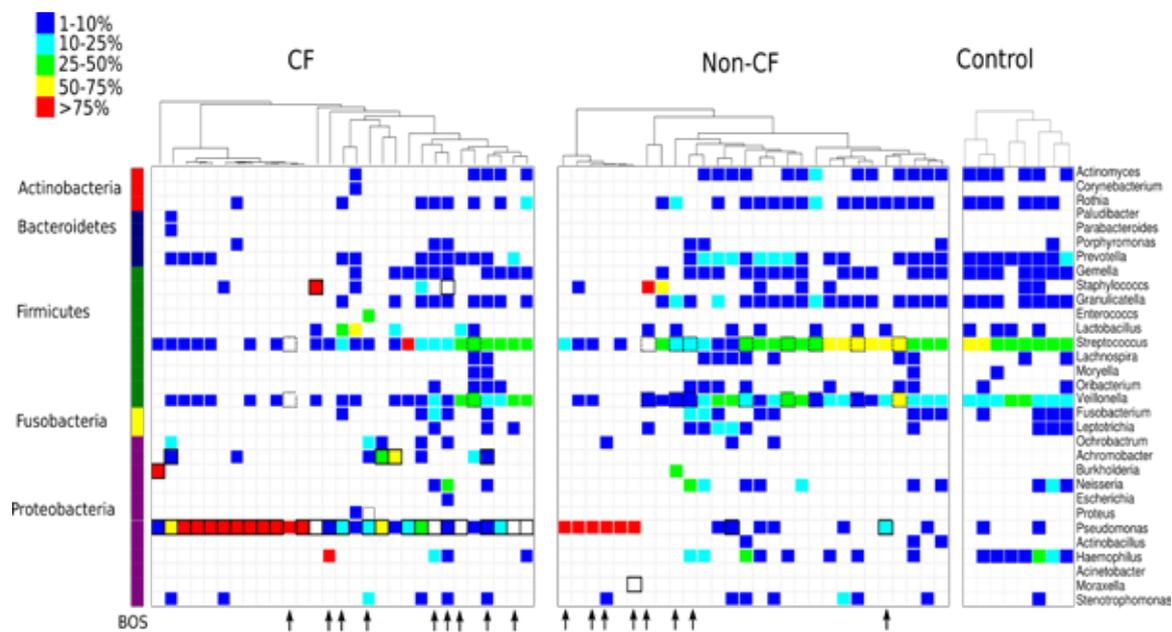
Figure 1 Bacterial community profiling using high throughput DNA sequencing of a highly conserved bacteria-specific gene (*16S rRNA gene*). In this example the microbial community composition in bronchoalveolar lavage samples obtained from lung transplant patients and controls is shown. Samples are clustered based on weighted Unifrac distance. Phylum level taxonomy is presented on the left and genus level taxonomy on the right. Arrows identify samples from individuals with bronchiolitis obliterans syndrome (BOS—the commonest form of chronic rejection). Solid black outlined boxes in the heat map represent organisms identified in pre-transplant sputum culture. In this example, loss of *Pseudomonas* from the allograft microbiome is associated with BOS in cystic fibrosis (CF) (P<0.01, exact logistic regression). Reprinted with permission of the American Thoracic Society from Willner DL, Hugenholtz P, Yerkovich ST, *et al.* Reestablishment of recipient-associated microbiota in the lung allograft is linked to reduced risk of bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2013;187:640-7. Copyright © 2014 American Thoracic Society.

ecosystem. In the case of the human microbiome that ecosystem is us. Traditional microbiological methods have been blind to the majority of the microbiome for the simple reason that most microorganisms have not been readily grown in the laboratory and thereby become amenable to manipulation and interrogation at the human-scale. Things began to change in the 1980s when it was recognised that microorganisms could be catalogued by their gene sequences, primarily the highly conserved 16S rRNA marker gene, without the need to grow them (1). This was the birth of culture-independent molecular microbiology. First, bulk nucleic acids are extracted from a biological sample and the target gene is PCR-amplified from the extracted DNA.16S rRNA is so conserved that it is possible to target all cellular lifeforms using "universal" PCR primers, which allows essentially all microorganisms in a given sample to be amplified in one PCR reaction. The mixture of 16S rRNA amplicons were then historically separated by cloning into *E. coli*, but now are sequenced in parallel using cloneless

next generation sequencing technologies such as 454 pyrosequencing or Illumina sequencing. The 16S rRNA amplicon sequences are then quality trimmed, compared to each other and typically clustered into operational taxonomic units (OTUs) and finally identified against a reference database. The number of reads assigned to each OTU for a given sample provides relative abundance information to create a community profile, an example of which is provided in *Figure 1*. Through such cataloguing surveys, it quickly became apparent that most microbial (evolutionary) diversity, roughly 85%, was not captured by our collection of domesticated microbial isolates (2). Moreover, in many instances, microorganisms isolated from a given habitat were, at best, bit players in ecosystem function (3). Combining 16S rRNA microbial profiling with 'metadata' such as disease state, physiology, etc., has proven to be a powerful way of identifying statistically significant correlations between the microbiome and disease that can be used for example as diagnostic biomarkers (4).

Today, gene-based surveys are still heavily used in microbiome research, but they have also evolved into genome-based (metagenomic) surveys courtesy of greatly improved sequencing and computational technologies (5). Taking a census of the total genetic inventory of a given ecosystem not only tells us who is there, but also what they are potentially capable of doing. Complementary molecular methods such as metatranscriptomics and metaproteomics, which identify mRNA transcripts and proteins respectively, can tell us which genes and pathways are being expressed under a given set of conditions (5). Consequently our understanding of the microbiome in humans has exploded in recent years (6).

The 'normal pulmonary microbiota'—does it exist?

Even today most medical students are taught that the lung is sterile. This idea was founded on data obtained in an era when non-selective genomic techniques were unavailable, so that microbial identification relied on basic general culture systems that were blind to 99% of bacterial species. Community profiling and metagenomic approaches now facilitate comprehensive characterization of the human microbiota and analysis of its role in health and disease. Given the extraordinary ability of microbes to adapt to even very hostile environments it seems unlikely that complete sterility of the lower respiratory tract that is continually exposed to the environment could be maintained, at a reasonable energy cost, given the enormous microbial load delivered with each breath. Although a number of studies purporting to confirm the presence of a healthy pulmonary microbiome have been published (7-10), all are in some way (as openly acknowledged by the authors) methodologically deficient either due to the acquisition of lower respiratory tract samples bronchoscopically (7-9), where oropharyngeal contamination is impossible to exclude, and/or due to the acquisition of lower respiratory tract material from potential organ donors (10) or lung resections (10). Organ donors will have invariably experienced gastric aspiration and/or ventilator associated bacterial colonization/infection (11), and lung resections from healthy subjects are difficult to obtain. A further difficulty is that the normal pulmonary microbiota, should it exist, will be many log lower than the surrounding human biomass, complicating DNA amplification and sequencing. Despite these deficiencies, the emerging model is that the human respiratory tract is not neatly compartmentalized into upper and lower tracts and hence the question of lung sterility is not a binary one. Intermittent colonisation of the lower respiratory tract occurs, even in healthy individuals,

with more persistent or permanent colonisation being common in certain scenarios. Dickson *et al.*, recently borrowed the 'adapted island model' from ecology to describe this pulmonary biogeography (12). While beyond the scope of this review, their work provides a convincing and useful framework for understanding lung ecology, and provides an intriguing perspective from which to view pneumonia. In their proposed model pneumonia does not occur as the result of a large inoculum of a pathogenic species overwhelming host defences, but as a small but snowballing disruption in the complex adaptive lung microbial ecosystem (12). A COPD exacerbation could be viewed in the same way. In summary, while these new models provide a more realistic framework for understanding the interactions between the human lung and the respiratory tract microbiota, the fundamental question remains whether this interaction is so intimate as to be classified as mutualistic or symbiotic.

Confirmation of such a relationship would imply benefit for both the microbiota and the host and would carry profound implications for our understanding of lung health. At the most basic level, we would need to reconsider what 'self' means in the context of lung immunology. In the best studied human system—the gut—it is clear that a healthy microbiota is critical to the development of both local and systemic immune responses and the maintenance of epithelial integrity. Perturbations in this symbiotic relationship ('dysbiosis') have been implicated in the pathogenesis of inflammatory bowel disease and the metabolic syndrome (13,14). Much of the literature on COPD microbiology has been written with the idea that the now colonised/infected lung was previously sterile. It is intriguing to think that a normal pulmonary microbiota may exist and that the act of its displacement by other organisms may in itself be detrimental to host health. In the lung, dysbiosis could predispose the host to excessive immune activation and/or loss of epithelial integrity—key features of multiple lung diseases including asthma, COPD and idiopathic pulmonary fibrosis. It could be that cigarette smoke induced dysbiosis, or disruption of lung biology by cigarette smoke, in conjunction with host genetic factors, may be important in COPD pathoegenesis, and, as a corollary, that restoring the microbiome could improve host health, opening the door to more subtle and more nuanced, but potentially highly effective, therapies.

What's known about the microbiome in COPD?

Only a handful of studies have been published exploring

the role of the lung microbiome in COPD and therefore our understanding this interaction remains in its infancy. Due to the heterogeneity of COPD features, particularly as they relate to disease severity, various studies have sought to evaluate the lung microbiome in stable or exacerbating disease states, in healthy patients *vs.* those with COPD, or in COPD compared to unrelated lung diseases such as cystic fibrosis (CF) or asthma. They have assessed the microbiome from differing parts of the airways and via different sampling techniques. As a result of these issues, the data that has resulted are not easily compared with each other and lack consistency.

Nevertheless these studies have produced valuable associative data on the changes in the microbiome in COPD. The major phyla that are associated with the normal lung microbiome appear to be Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, and most studies agree that this is regardless of the presence of COPD, its status or severity (8-10,15,16). These studies have demonstrated no apparent difference in the total bacterial load nor in diversity. However, principle component analyses have revealed that the microbiome of COPD patients clusters separately (and therefore differs) from that of healthy controls. This is partly driven by increases in members of the phylum Firmicutes in patients with stable COPD (8,10,15,16). In severe COPD, however, the microbiome is unlike that of mild disease. In two notable studies using lung explants (2,3) and one using sputum (17) from severely affected COPD lungs compared to healthy controls, a shift was observed to a decrease in lung microbiome diversity driven partly by increases in Proteobacteria. This correlated with a shift towards the dominance of particular bacterial genera, especially *Pseudomonas* although one must consider that this effect could be influenced by bacterial induced exacerbations. Three studies have demonstrated a decrease in diversity during exacerbations and with antibiotic treatment and a further decrease in diversity with increasing severity and persistence of these disease flares (17-19). As a majority of all COPD exacerbations are associated with respiratory infections (20) this finding is not surprising. Nevertheless, no study has definitively shown that a reduction in COPD microbiome diversity in the lung has caused the outgrowth of a particular bacterial genera or an increase in bacterial load. This is difficult to assess in humans but may be investigated using animal models that are representative of the human condition (21-24).

One important issue with investigations thus far is sample collection. Cabrera-Rubio *et al.*, assessed the microbiome from bronchial aspirates, sputum, bronchial lavage (BAL) and bronchial mucosal brushings. They found that the microbial diversity was lower and the load was higher in samples collected from the upper (aspirate and sputum) compared to the lower respiratory tract (BAL and mucosa). Furthermore, Erb-Downward *et al.*, found that the microbial community in tissue from lung explants differed depending on the location from which the sample was taken, even within same patient (8). This indicates that the COPD lung microbiome is not homogeneous.

Recently it has become clear that inhaled corticosteroid use in COPD is associated with an increased risk of pneumonia (25,26). At present this observation, whilst very robust, remains empiric with little understanding of the mechanisms that lead to pneumonia, who may be at highest risk, and how that risk may be mitigated in the face of continued drug exposure. It is likely that answers to these highly relevant and clinically important questions will come through the deeper understanding of bacterial ecology in the COPD lung which is now obtainable with ecogenomic approaches (19).

Host-pathogen interactions in lung disease

Whilst exposure to environmental irritants, particularly cigarette smoke, is obviously central to COPD pathogenesis, airway infection also plays a role both in exacerbations and in disease progression even during the 'stable' phase of the illness (27). However, not all bacteria are created equal in this regard. For the best-studied pathogen, *Haemophilus influenzae*, it is clear that some strains induce more inflammation (particularly IL-8 induced neutrophilic inflammation) than others (16). However, given that most of the literature in this field was developed using traditional microbial techniques, the roles and/or impact of bacterial ecosystem was not incorporated into these studies.

When a host encounters a potential threat, annihilation of the threat is only one of the defence strategies available to ensure survival. In some cases, attempted annihilation may do more harm than good through collateral damage to host tissues. Other than this traditional concept of 'resistance' the other strategies available to the threatened potential host include 'avoidance' of the threat prior to infection as well as 'tolerance' (28). In the lung, the mechanisms of resistance (e.g., the innate and adaptive immune responses) and avoidance (e.g., nasal hairs, the cough reflex and the mucociliary escalator) are well described, but the concept of tolerance is poorly studied. Nevertheless, we see examples of tolerance in our thoracic clinics every day.

For instance, patients with CF live for decades with a

microbial load in the lower respiratory tract that would probably be fatal to most hosts. Tolerance to infection, or so called 'disease tolerance' does not represent a failed eradication effort, but rather a highly specific host defence strategy which minimises the negative health effects of infection, without directly affecting pathogen burden (28). Chronic *Pseudomonas* infection in the CF lung is an example and is associated with classic features of the tolerant state, including tolerance to endotoxin (29) and polarisation of circulating monocytes toward an M2 phenotype. These cells are characterised not only by reduced secretion of pro-inflammatory cytokines like TNFα and secretion of anti-inflammatory cytokines like IL-10, but also an impaired capacity to present antigen despite markedly enhanced phagocytic activity (29). Recently, as would be predicted from the disease tolerance model, multiple investigators have noted that host health is dissociated from *Pseudomonas* biomass in CF (30-32), and similar observations have been made in lung transplant recipients (33). Recently, and again in the setting of lung transplantation, we have demonstrated that tolerance to *Pseudomonas* translates into allograft tolerance (*Figure 1*) (34). It is likely that gaining a better understanding of the tolerogenic mechanisms in operation at the interface of the airway mucosa and the role of commensal bacteria will provide important insights into COPD pathogenesis, and potentially guide the development of new treatments. This will depend heavily on accurately describing commensal microbial ecology in the COPD lung, an aim now achievable through the application of metagenomic techniques.

The microbiome in COPD—is it only about the lung?

Most microbiome studies to date have involved the gastrointestinal (GI) tract and diseases at that site such as colitis. Yet it is increasingly being recognised that changes to the GI microbiome can have profound effects on extraintestinal organs.

One of the earliest observations has been the effect of antibiotics on obesity. It has long been known that antibiotics can lead to increased weight gain in farm animals and have been used as growth factors in standard practice in many countries since the 1950s (35). This observation has been closely investigated and links have been shown between antibiotic use and obesity in mice (36), and with weight gain in malnourished humans (37). Metagenomics of the gut microbiome in murine studies has revealed that antibiotics cause a shift in microbial populations to those capable of producing short chain fatty acids as metabolites.

These metabolites have been associated with improvements to colonic and systemic health. Furthermore the antibiotics caused an upregulation of liver enzymes involved in lipogenesis and triglyceride synthesis (36). Other studies have demonstrated gut microbiome effects on diabetes and atherosclerosis through alterations in the metabolites produced by the microbes that mediate communication between the microbiome and its host (38,39).

To date, the involvement of the microbiota in lung health has only been inferred through associative studies. There are no studies that demonstrate a direct effect of changes to the lung microbiome causing a subsequent change to lung health. This is in part due to the field's infancy, and because the microbial load in the lung is low and the potential for contamination with microbes from the oral cavity and/or nasopharynx during sampling is high. However, studies are emerging that highlight the involvement of the gut microbiome in maintaining lung health and in contributing to lung disease. Ichinohe *et al.*, demonstrated that mice treated with oral antibiotics had diminished immune responses when subsequently infected with influenza (40). Specifically, treated mice had significant reduced influenza antibody titres and CD4 and CD8 T-cell responses. In another study, Russell *et al.*, demonstrated that neonatal mice given oral vancomycin had increased immune responses in the lung when challenged with ovalbumin in a model of allergic asthma. These mice also had increased airway hyperresponsiveness compared to untreated mice (41). Complementary to this, Ong *et al.*, were able to demonstrate a strong association in children given antibiotics in the first year of life with the development of both transient and persistent asthma. These associations were strong even when children who received antibiotics for respiratory tract infections were excluded (42).

In regard to COPD, it has been known for many years that smoking impacts intestinal as well as lung health (43). Furthermore, there is a strong association between inflammatory bowel diseases and COPD, with many patients are also affected with Crohn's disease (44,45). Moreover, smoking and smoking cessation have been recently shown to have clear effects on the microbiome of the gut (46). Thus, the evidence suggests that smoking can affect gut microbiota which in turn may induce systemic effects.

Conclusions & future directions

Since bacterial colonisation and infection is common in COPD and is central to the pathogenesis of exacerbations, gaining a more comprehensive understanding of lower

respiratory tract bacterial ecology in patients with COPD is likely to be of considerable importance. The tools to achieve this objective are now readily available, but in order to make sense of the findings in stable COPD, the more fundamental question 'do we have a normal lower respiratory tract microbiota?' will need to be answered. It is also apparent that accurate determination of the makeup of the lower respiratory tract microbiome is confounded in studies where access to the lower respiratory tract is gained via the oropharynx where the bacterial biomass is high, even if a protected brush is used (47). Animal studies and approaches to obtaining human material surgically (e.g., at the time of lung resection and transplantation) can circumvent this difficulty. In the future, determining the makeup of the microbiome in healthy smokers and patients with mild COPD will assist in determining whether dysbiosis is a triggering event for COPD progression or whether it is a biomarker of more severe disease. Furthermore, it is likely that non-bacterial microbes will contribute to COPD pathogenesis, so determining the makeup of the viral and fungal microbiomes (the 'virome' and 'mycobiome'), alongside the bacterial microbiome, in COPD will also be key objectives. These studies will complement, and may, in terms of their impact on the practice of medicine, even outshine the findings of the genomic era which began with the sequencing of the human genome (48).

Acknowledgements

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Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings

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Abstract: Respiratory and/or limb muscle dysfunction, which are frequently observed in chronic obstructive pulmonary disease (COPD) patients, contribute to their disease prognosis irrespective of the lung function. Muscle dysfunction is caused by the interaction of local and systemic factors. The key deleterious etiologic factors are pulmonary hyperinflation for the respiratory muscles and deconditioning secondary to reduced physical activity for limb muscles. Nonetheless, cigarette smoke, systemic inflammation, nutritional abnormalities, exercise, exacerbations, anabolic insufficiency, drugs and comorbidities also seem to play a relevant role. All these factors modify the phenotype of the muscles, through the induction of several biological phenomena in patients with COPD. While respiratory muscles improve their aerobic phenotype (percentage of oxidative fibers, capillarization, mitochondrial density, enzyme activity in the aerobic pathways, etc.), limb muscles exhibit the opposite phenotype. In addition, both muscle groups show oxidative stress, signs of damage and epigenetic changes. However, fiber atrophy, increased number of inflammatory cells, altered regenerative capacity; signs of apoptosis and autophagy, and an imbalance between protein synthesis and breakdown are rather characteristic features of the limb muscles, mostly in patients with reduced body weight. Despite that significant progress has been achieved in the last decades, full elucidation of the specific roles of the target biological mechanisms involved in COPD muscle dysfunction is still required. Such an achievement will be crucial to adequately tackle with this relevant clinical problem of COPD patients in the near-future.

Keywords: Muscle dysfunction; deconditioning; hyperinflation; systemic inflammation; proteostasis

For the last one and a half million years, our species has been moving and breathing on earth (1). In addition, we have been able to modify our environment, develop tools and skills and, thereby, create what today we call culture. All these functions essential for both individual survival and the biological success of our species are linked to the contractile properties of striated muscles. Their name derives from their structure, since their basic functional unit, the sarcomere, gives them a typical striped appearance. When, as a result of a disease or even a physiological process (such as ageing) our muscles do not work properly, we become weak and frail and may even die.

Different muscles, different tasks

However, the tasks of the muscles are not homogeneous. Some of them, such as those located in the pelvic girdle and lower limbs are specialized in enabling us to move around. Whereas the muscles located in the upper limbs and scapular girdle are essential for the manipulation of all kinds
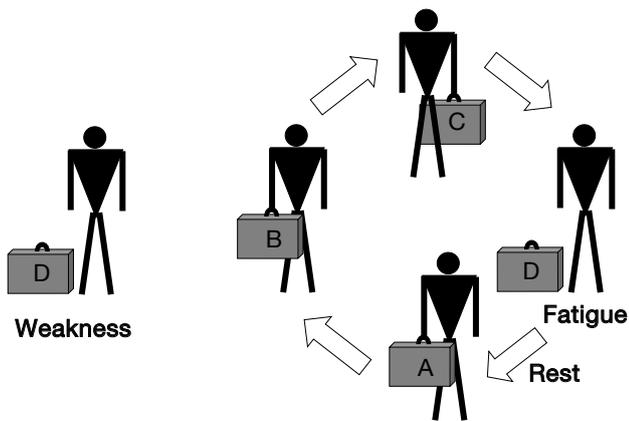
Figure 1 Schematization of weakness (left) and the process of muscle fatigue (right). (A) The task is carried out by a specific muscle group; (B) when this group comes close to task failure due to muscle fatigue; (C) the workload is shifted to complementary muscles which can also sustain it; (D) finally, they also become fatigued and task failure occurs. However, the duty can be reinitiated following a period of rest.

of objects and also for self-care activities (2,3). Both the upper and lower limb muscles are also known as "peripheral" muscles. Like those located in the trunk they are also referred to as "skeletal muscles" since they move the bones by means of their joints, thus producing movement and/or maintaining the skeleton structure. Other striated but very specialized muscles, whose role is to provide the subject with alveolar ventilation through breathing movements, are known as respiratory muscles. The latter can be subdivided into either inspiratory or expiratory muscles, depending on the part of the ventilatory cycle (inspiration or expiration, respectively) where their activity is more predominant. Inspiratory muscle contraction generates changes in thorax shape and volume, which combined with natural retraction of the lungs, increases the negativity of intrathoracic pressure. The gradient between intrathoracic and atmospheric pressures determines the airflow to the lungs. The main inspiratory muscles are the diaphragm (which can be subdivided into costal and crural portions), external intercostals and parasternals (4-10). However, many other muscles such as scalenes, sternocleidmastoid, latissiums dorsi, serratus and pectoralis can also participate progressively in the breathing effort if ventilatory loads or demands increase and/or the main inspiratory muscles fail to perform their job properly (11-14). Under normal conditions, expiration is a much simpler process since

the mere relaxation of inspiratory muscles results in the reduction of the negativity of pleural pressure and a slightly positive alveolar pressure. This gives rise to the exit of the air from the respiratory system. However, some muscles can facilitate exhalation if necessary, and are therefore known as expiratory muscles (9,15-18). The main expiratory muscles are those that make up the abdominal wall (mostly major and minor obliques and transverse abdominis) as well as the internal intercostal group, with the exception of parasternals (which as mentioned before act predominantly during inspiration). Finally, another very specialized and critical muscle is the myocardium. Its contraction is responsible for blood flow through the entire body, including not only the perfusion of peripheral tissues but also pulmonary circulation, which is essential for gas exchange. However, its function has become so specialized that the muscle phenotype has diverged from that of classical striated muscles, to the point where it is classified as a different category: the cardiac muscle.

Muscle function and dysfunction

Striated muscles have two main functional properties: strength, or the ability to develop a maximal effort, and endurance, or the ability to maintain a submaximal effort through time. Therefore, it is worth noting that the time-dependence (shorter for strength and longer for endurance) and the magnitude of the effort (maximal in the case of strength and submaximal in that of endurance) are the two main differences between these concepts. Muscle dysfunction can be defined as the situation where skeletal muscles show reduced strength and/or reduced endurance, being unable to perform their physiological tasks adequately. Muscle dysfunction can be expressed both as fatigue or weakness. Fatigue is a state in which the muscle is temporarily unable to perform its current tasks (*Figure 1*). This condition is reversible with rest, thus differing from the concept of weakness, which is a much more permanent impairment in muscle contractile properties. Although fatigue and weakness appear to be very different conditions, they are related in the sense that a weak muscle becomes more easily fatigued. There are different conditions, and not only diseases, which can result in muscle dysfunction. Some involve striated muscle structure directly while others primarily affect the structures of the nervous, vascular and osteoarticular systems (18,19).

When lower limb muscles become ineffective in doing their tasks, the mobility of the individual is reduced. This

in turn generates the perception of being disabled, and has an extraordinary impact on one's quality of life. A similar effect results from the loss of function in the upper limb muscles, since subjects would not only be unable to maintain their professional lives but, at advanced stages, they would also require assistance even for the simplest of everyday tasks. If the respiratory muscles fail to perform their tasks, hypoventilation occurs and subsequently, oxygen is deficiently provided to the different tissues (including the striated muscles themselves). Therefore, aerobic metabolism becomes impaired and anaerobic pathways increase their activity, which has consequences for both energy generation and acid-base homeostasis. The latter can even become aggravated by the fact that the drop in ventilation leads to carbon dioxide retention.

The analysis of cardiac muscle dysfunction is very complex and lies beyond the scope of this review, which will focus on the functional impairment of both limb and respiratory muscles in COPD.

Chronic obstructive pulmonary disease (COPD)

This is a highly prevalent respiratory disease, with enormous costs for both health and social care systems. The main cause of COPD is tobacco smoking, although other factors can also be involved (20,21). Inhalation of noxious particles suspended in the smoke results in airway and lung inflammation, as well as in the destruction of lung parenchyma, which are directly related to the occurrence of the two main entities included in COPD: chronic bronchitis and pulmonary emphysema. COPD has been classically defined in terms of its impact on lung function, mainly characterized by a non-fully reversible airflow obstruction (20), together with pulmonary hyperinflation and gas exchange abnormalities. Although symptoms such as coughing and breathlessness appear slowly, they progress and eventually lead to exercise limitation and death. However, in recent years it has become evident that COPD is not only a pulmonary disease since many of the symptoms are caused by the involvement of other organs and systems (22). This systemic involvement includes abnormalities in skeletal muscles, blood, nervous system and even in the bone metabolism (22-26), but its causes and mechanisms remain unclear. It is believed, however, that systemic inflammation (directly linked or not to the local inflammatory process already present in the lungs), plays a key role in the occurrence of extrapulmonary manifestations of COPD (20,22,25). Although systemic inflammatory

response syndrome (SIRS) is an expression which is usually restricted to multiple organ damage and subsequent dysfunction appearing in the course of sepsis (27-29), some authors have suggested that this term or the alternative chronic systemic inflammatory syndrome (CSIS), can also be used in other entities, such as COPD (30,31), which are characterized by the presence of low level but persistent systemic inflammation and multiple organ involvement.

Muscle dysfunction in COPD

This is probably the most extensively studied systemic manifestation of COPD and can involve both respiratory and peripheral muscles (24). It is considered to be of multifactorial origin, with local and systemic factors interacting to modify, in different ways, the phenotype and function of any specific muscle (*Figure 2*) (32). The following pages summarize the current knowledge regarding the status of different striated muscles in COPD patients. They include structural, metabolic and functional findings as well as a review of the factors, which have been involved in muscle dysfunction.

Respiratory muscles in COPD

From the 1970s it has been well established that the function of the diaphragm deteriorates in subjects with pulmonary emphysema (33,34). This is mainly due to the dramatic increase in lung volume known as pulmonary hyperinflation (*Figure 2*), which shortens and flattens the diaphragm and negatively modifies its length-tension relationships. As a result, the diaphragm loses its capacity to develop contractile force (35). In addition, the involvement of the airways, inherent to COPD, implies that respiratory muscles have to cope with increased airway resistance and airflow obstruction. Both pulmonary hyperinflation and increased airway resistance increase the work of breathing, which is mainly dependent on inspiratory muscles. In other words, from a mechanical point of view, respiratory muscles need to perform heavy duty under very adverse conditions. Likewise, and from a metabolic point of view, although the nutrient and oxygen demands of respiratory muscles are relatively low under normal conditions (36), in COPD patients they become progressively higher as a consequence of their increased tasks. This is particularly important in individuals whose oxygen delivery to these muscles can be easily compromised by simultaneous gas exchange abnormalities occurring in the lungs. Therefore, respiratory muscles in COPD are also exposed to a potential metabolic
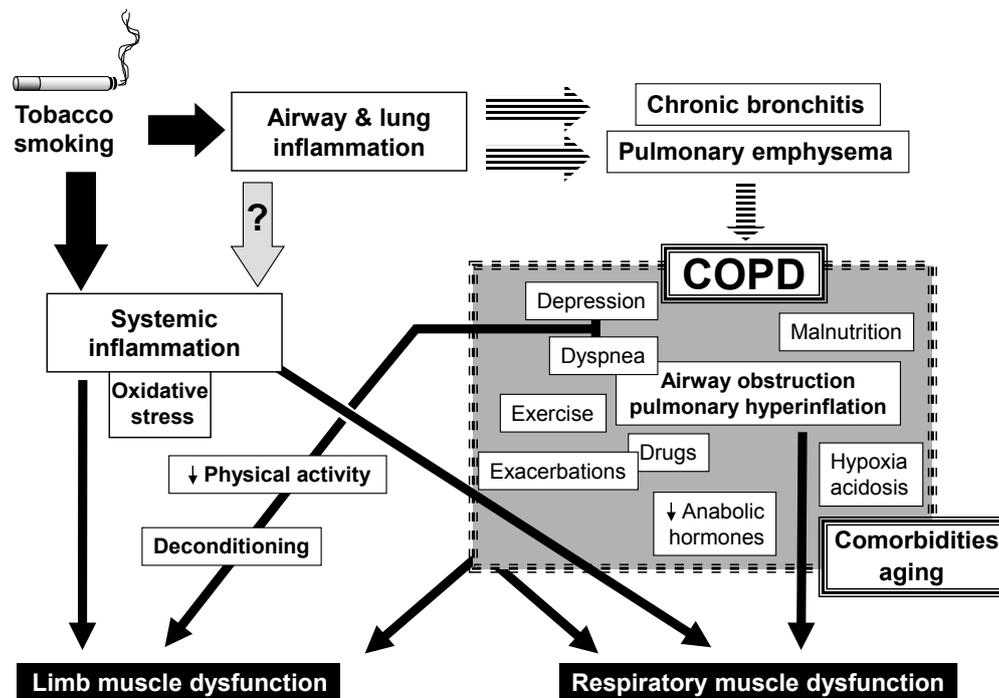
Figure 2 Consequences of tobacco smoking include local (pulmonary) and systemic inflammation. The former will lead to pulmonary changes characteristic of COPD, whereas the latter appears to be linked to its extrapulmonary manifestations including muscle dysfuntion. However, the key deleterious etiologic factors for muscle dysfunction are pulmonary hyperinflation for respiratory muscles and deconditioning for limb muscles. COPD, chronic obstructive pulmonary disease.

imbalance between offers and demands (32).

Nevertheless, respiratory muscles are not only subject to local mechanical and metabolic factors directly deriving from changes in the airways and lung parenchyma. Like other striated muscles in the whole body they can also be influenced by systemic factors such as inflammation and oxidative stress (both of which have been detected in the blood stream of COPD patients), nutritional depletion and the effect of certain drugs used in the treatment of this condition (*Figure 2*) (37-42). These systemic influences will be more extensively reviewed in the following sections. Moreover, as a result of all these local and systemic factors, molecular and cellular phenomena such as focal inflammation, oxidative stress and epigenetic changes (43-45) will be present in the diaphragm and rib muscles of COPD patients.

Unexpectedly and despite all these negative factors, the final result is not so negative. Certainly, the diaphragm and other respiratory muscles show an impairment in their functional properties (33,46,47). However, it has been shown that the diaphragm of COPD patients is able to develop even greater strength than that of healthy subjects when both are forced to maintain similar levels of hyperinflation (34).

To explain such a paradox it is important to remember that striated muscles are very sensitive to modifications in their environment, as they are extraordinarily capable of changing their phenotype to adapt to the ongoing conditions. In keeping with this, different authors have supplied evidence that respiratory muscles actually undergo structural and metabolic changes, which would partially explain the paradox of their relatively preserved function. Phenotypic changes occurring in the respiratory muscles of COPD appear to include modifications in the expression of structural proteins such as myosin heavy chain (MyHC) isoforms, mitochondrial and capillary content, sarcomere length and fiber type proportions and sizes (48-56). Moreover, some experimental models suggest that these adaptive phenomena might be directly linked to chronic increase in respiratory loads (57), and they appear to be mediated by the occurrence of transient muscle damage (58,59), which would be followed by muscle repair/regeneration and remodeling (*Figure 3*) (57). However, coexisting with adaptive changes, and in addition to muscle damage, there are also indications that myopathy may be present in the respiratory muscles of COPD patients (60). Therefore, it is not so surprising that
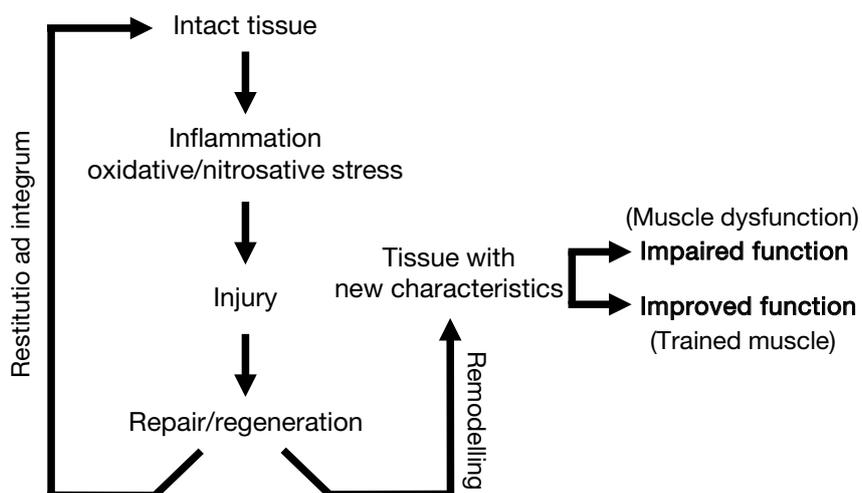
Figure 3 Process of tissue regeneration and remodeling following an insult.

isolated fibers from their diaphragms have been shown to develop less force than those from control individuals (61).

In contrast with the relative abundance of data on inspiratory muscles, information regarding changes occurring in expiratory muscles of COPD is in short supply. This is somewhat surprising since expiratory muscle function appears to be important in COPD patients for both coughing and breathing (62-66), but it is deteriorated (67-69). This expiratory muscle dysfunction, in contrast to what occurs in inspiratory muscles, cannot be attributed to mechanical changes occurring in the lungs. In this regard, hyperinflation might even improve the length-tension relationships of abdominal expiratory muscles (70). Neither can it be ascribed to muscle deconditioning, as is considered the case for peripheral muscles (see next sections). Therefore, systemic factors leading to molecular and cellular abnormalities are the most probable culprits in expiratory muscle dysfunction occurring in COPD. From the very sparse reports available it appears that fiber phenotype is altered in these muscles (54) but their global metabolic properties appear to be maintained (71).

Peripheral muscles in COPD
This is a very heterogeneous group of contractile elements located in the upper and the lower limbs, which perform different tasks including walking and the manipulation of instruments. Although the function of both upper and lower limb muscles can be impaired in COPD patients (24,32,47,72,73), the level of dysfunction is not necessarily the same. In fact, leg muscles appear to be more severely affected than those located in the upper limbs (69,74). The

reason for these differences is believed to be closely related to the differential activity of these two groups of muscles in COPD patients.

Lower limb muscle dysfunction is not merely a local problem since it has a direct impact on the exercise capacity of the patients (72,75-78). It is characterized by a reduction in both muscle strength and endurance (24,47,72,73,79), as well as an impairment in the efficiency of muscle metabolism, since lower limb muscles of COPD patients consume more oxygen for any particular workload and are characterized by an early and increased production of lactate (78). Regarding structural and metabolic findings, most of the studies have been performed in the vastus lateralis, which is a part of the quadriceps muscle. These studies have shown overall muscle mass reduction, along with smaller fibers, and a "less aerobic phenotype" (reduced percentage of oxidative fibers and MyHC-I, fewer blood vessels and capillary contacts per fiber, a reduced myoglobin content and a diminished enzyme capacity in the oxidative pathways) (53,80-89). Moreover, the fact that the oxidative capacity of the muscle is reduced, whereas the oxygen delivery is relatively preserved in COPD, supports the existence of an inefficient intracellular use of this gas (78,90). This probably explains the above mentioned early lactate production and myocyte acidification (78,87,91,92). In contrast to abnormalities in oxidative pathways, the activity of glycolytic tracks is maintained or even increased in these patients (83,87).

Although studies focused on the upper limb muscles are much less frequent than those dealing with lower extremities, the findings are consistent with the relative

maintenance of histological, biochemical and functional properties in the former (69,74,93-96). Furthermore, some of these muscles show mild adaptive changes, similar to those exhibited by respiratory muscles, along with certain other modifications that are more characteristic of lower limb muscles. Deltoid muscle, for instance, shows the coexistence of different fiber size subpopulations (normal, hypertrophic and atrophic) (93), preserving the percentages of different fiber types as well as the enzyme activities in the aerobic pathways (94). The brachial biceps, on the other hand, show an unchanged fiber type composition along with a mild decrease in size as well as preserved behavior in the aerobic pathways (95,96). The final result of all of these cellular and molecular changes is a generally mild-to-moderate reduction in the functional properties of the upper limb muscles, which has a lower impact on the activities and life of the patients than lower limb muscle dysfunction (69,74).

The causes of peripheral muscle dysfunction have not been completely elucidated, but it is generally accepted that at least for the lower limbs, muscle deconditioning, resulting from a reduction in physical activity, plays a key role (*Figure 2*) (24,32,47). However, since most of the functional and structural limb muscle changes are only partially reversed by muscle training, other intrinsic and systemic factors are probably also implicated. Many of them would be common for all the striated muscles throughout the body.

To sum up, changes shown by different skeletal muscles in COPD patients are very heterogeneous, depending on the muscle group being judged. This indicates that these changes are most probably the result of the complex interaction of different factors, with each one being unique for any particular muscle (97). In the following paragraphs, the systemic factors and cell-molecular mechanisms that have been involved in the pathogenesis of muscle dysfunction in COPD will be briefly reviewed.

Systemic factors involved in muscle dysfunction in COPD

Inflammation

This can be considered as either a systemic or a local factor since inflammatory activity has actually been demonstrated both in different solid tissues such as skeletal muscles and in the blood of COPD patients (37,98-101). It is believed that the initial tobacco and/or other pollutants insult crosses through the alveolus-capillary interface and then immediately spreads through the systemic bloodstream targeting different organs (102). Alternatively,

or complementarily, other authors sustain that the initial inflammatory process induced by these deleterious factors in the airways, lung parenchyma and pulmonary vessels is later disseminated through systemic circulation reaching different target-organs including muscles ('spill over' theory) (99,103-105). However, the absence of concordance between the inflammatory markers found in the blood and in other tissues, and the occasional occurrence of systemic manifestations preceding clear lung involvement strongly argues against the latter theory (42,106,107). Whether by one way or the other, it is generally accepted that chronic systemic inflammatory signal and the subsequent multi-local inflammatory activity are significant contributors to muscle dysfunction occurring in COPD (24,32,42,47). Among the evidence for the persistent systemic inflammatory signal there are studies which show increases in the serum levels of C-reactive protein (CRP), fibrinogen and different proinflammatory cytokines (37,99,101) as well as different abnormalities in circulating white cells (25,99,108). Similarly, inflammatory activity has been documented in other extrapulmonary targets such as striated muscles. In this respect, an increase in inflammatory cells has been documented within the peripheral muscles of COPD patients (100), although other authors have been unable to confirm such findings (109). In addition to cellular changes, an increase in the expression of local proinflammatory cytokines has been described in respiratory and peripheral muscles of COPD patients (100,110), although again not all the authors agree with this finding (98). It is well known that these inflammatory mediators are capable of inducing an increase in the degradation of intracellular proteins either through direct activation of proteolytic pathways or the development of oxidative stress (111,112).

One important aspect is the probable impact that exacerbations have on the levels of systemic inflammation in COPD. Some authors have demonstrated that the inflammatory load already present in the bronchial tree of the patients can be further increased by colonization or infection by microorganisms (113), which in turn may lead to exacerbations (114). This factor would support the use of local anti-inflammatory or antibacterial drugs to reduce "the inflammatory overload" present in the lungs. However, the extent to which this local 'over inflammation' is reflected in extrapulmonary targets such as skeletal muscles is still unclear.

Oxidative and nitrosative stress

Reactive oxygen species (ROS), a product of the oxygen metabolism, and nitric oxide (NO) are normally present

in skeletal muscles. Moreover, a moderate level of ROS, which are provided by the mitochondrial respiratory chain pathway and some microsome enzymes, is necessary for excitation-contraction coupling and an appropriate muscle contraction (115). The same is true of NO (synthesized by specific enzymes in both fibers and endothelium), which plays a role in myoblastic differentiation, carbohydrate metabolism, blood flow regulation to the fibers and electromechanical muscle coupling (116,117). However, when there is an increase in the production of oxygen or nitrogen reactants and/or their scavengers are unable to buffer them, oxidative and/or nitrosative stress occur. This leads to structural damage in proteins, lipids and DNA, with important functional consequences (118-120). Interestingly, the production of free radicals is modulated by a variety of factors including the presence of inflammatory mediators, blood supply and level of activity (121,122).

Free radical stress is believed to be involved in COPD pathogenesis. Moreover, as with inflammation, oxidative and nitrosative stress appear to extend beyond the lung to reach other systems. In this respect, these phenomena have been found both in animal models and COPD patients, where they involve both respiratory and peripheral muscles (43,123,124). Moreover, the oxidative stress level within the respiratory muscles appears to be directly related to the mechanical loads they have to deal with, and directly influences their function (43). Lower limb muscles in turn show even more stress than respiratory muscles (125), and the functional consequences (126) are probably related to changes induced in key enzymes such as creatine kinase and carbonic anhydrase (127). In this case, the local oxidative stress might be caused by the reduction in muscle activity, which is known to decrease the content of reduced glutathione, while increasing both oxidized glutathione and lipid peroxidation (128). However, the presence of all these deleterious phenomena can also open new therapeutic strategies for COPD patients, such as the use of antioxidants (126,129).

Deconditioning

Deconditioning is the result of the reduction in physical activity that, as previously mentioned, is frequent in COPD patients as a consequence of their ventilatory limitation, a sedentary life style and reactive depression. The effects of deconditioning are especially evident in lower limb muscles. There is strong evidence for the key role of deconditioning in limb muscle changes since many of the structural and biochemical changes observed in COPD are similar to those

induced by disuse (fibers become smaller and the proportion of type II fibers increases) (130), and are reversible with training (131). However, the fact that muscle dysfunction has been observed even in hand muscles (69), which are being continuously used even by very severe COPD patients, and that training does not completely reverse all muscle abnormalities (78,131), strongly suggests that deconditioning is not the only factor.

A particular case is the muscle dysfunction appearing in those COPD patients who have been submitted to mechanical ventilation, with or without complete sedation. This therapeutic procedure, with many different modalities, is characterized by absolute or relative muscle rest, which leads to muscle involution and dysfunction (132). However, in this specific case, disuse is not limited to limb muscles but also affects respiratory muscles (133,134). Furthermore, other factors common in critically ill patients, such as sepsis, malposition and drugs (27,134-136) can further deteriorate muscle function in COPD patients submitted to mechanical ventilation.

Nutritional abnormalities

Nutritional abnormalities expressed as body waste and changes in body composition are also frequently observed in COPD patients (40,41,137), with their prevalence dependent both on the variables analyzed and the population considered. Body mass index (BMI) is the most currently used nutritional threshold variable, since it is clearly related to life expectancy in COPD patients (138). However, this is a very general parameter that can lead to an underestimation of nutritional abnormalities, especially in women. Therefore, the fat free mass index (FFMI) has been proposed as a better and more sensitive alternative for classifying patients (139). Malnutrition associated with COPD can lead to reductions in muscle mass, changes in the proportions and size of muscle fibers (140), and muscle dysfunction (41). It has been attributed to different factors including the presence of systemic inflammation (37,42,47,101), a reduction in food intake (probably due to changes in leptin metabolism) (141), and an increase in metabolic cost derived from the increased work of breathing (which in turn is the consequence of the impairment in the mechanical properties of the ventilatory system) (42,142). The prevalence of nutritional abnormalities, however, does not appear to be homogeneous through different geographical areas, since it seems to be lower in Mediterranean countries than in Northern Europe and North America (143,144). These differences have been

attributed to life-style factors such as dietary habits and the level of physical activity (145).

Gas exchange abnormalities

Ventilation-perfusion mismatching present in COPD patients frequently results in chronic hypoxia, with or without hypercapnia. In addition, respiratory muscle dysfunction can also contribute to gas exchange abnormalities through the development of absolute or relative hypoventilation. Conversely, both chronic hypoxia and hypercapnia can have effects on muscle function. Hypoxia results in a reduction of muscle strength and endurance, contributing to exercise limitation (146,147). This loss of muscle function can be explained by the induction of systemic inflammation, oxidative stress and apoptosis, the imbalance between protein synthesis and catabolism (proteostasis), the limitation in the aerobic pathways and impaired muscle regeneration (148-151). Hypercapnia, directly or through the development of respiratory acidosis, may also induce an impaired muscle proteostasis and affect muscle contractile properties (152-154).

Tobacco smoking

It is well known that even nonsymptomatic smokers can exhibit fatigability and reduced muscle resistance (155,156). This can be well explained by the anorectic effects of tobacco, which may lead to the loss of muscle mass, as well as inducing inflammation, oxidative stress, an imbalance between protein synthesis and degradation in the muscle, and blockading the neuromuscular transmission (102,106,157-159).

Drugs

Some of them, such as systemic steroids, with very well known deleterious effects on muscle structure and/or function, are used relatively frequently in the treatment of COPD patients. Steroids can induce both chronic and acute myopathies (160). In fact even low doses of these drugs can cause the chronic form, characterized by weakness of proximal muscles, if taken during a relatively long period of time (161). Acute myopathy in turn appears a few days after steroid administration, and the symptoms do not predominate in a particular muscle group. Therefore, it is not surprising that corticosteroids have always been related to muscle dysfunction in COPD patients, since they even influence their survival expectancies (161).

Anabolic hormones decrease or inefficiency

Plasma levels of testosterone, a steroid hormone with important anabolic effects such as the increase in muscle protein synthesis (162), have been shown to be reduced in some COPD patients (163,164). This abnormality has been explained by the effects of smoking, hypoxia and drug therapies (24,165) but its functional implications remain unclear since both muscle strength and endurance appear to be preserved in such patients (164). In the case of the growth hormone, another powerful anabolic agent, the problem is not the plasma level but the interaction with the insulin-like growth factor (165,166), which is altered and can potentially impair proteostasis and reduce muscle mass leading to dysfunction (42).

Exercise

This factor is essential to muscle performance but should be kept between physiological limits. When exercise is too intense it can lead to the development of metabolic dysregulations, systemic inflammation and oxidative stress, muscle damage and inhibition in the expression of genes crucial for muscle mass maintenance (59,167-171). Therefore, it is not surprising that it can also contribute to muscle dysfunction (169). Moreover, some COPD patients show marked energetic-mechanical inefficiency during exercise (78,172,173). This could be the consequence of a reduced matching between the expression of genes linked to bioenergetics and those participating in programs of muscle regeneration and remodeling (174). Furthermore, not only the intensity of the exercise but its time course can influence the response of the muscle in COPD patients, mostly in those with reduced body weight. In this respect, high intensity training programs may induce oxidative stress in the patient's muscles during the first weeks (175) but this effect disappears if the program lasts longer (around 8 weeks) (176).

Exacerbations

The relationships between inflammation and infections present during exacerbations and muscle dysfunction have been previously analyzed. However, these acute episodes also involve other deleterious factors such as inactivity, negative energy balance and the use of systemic steroids (42,177-179). Therefore, it is not surprising that exacerbations are widely considered to be one of the factors that contribute the most to muscle wasting and dysfunction (24,32,42). Both develop early in the episode, and last for a relatively long time (42). Conversely, those patients with muscle dysfunction show an increased risk of hospital admission due to exacerbations (180,181).

Limb muscles	Respiratory muscles
Fiber atrophy	↑ % Type I fibers
↓ % Type I fibers	↑ Capillary contacts
↓ Capillary contacts	↑ Mitochondrial density
↓ Mitochondrial density	↑ Myoglobin
↓ Enzyme capacity	↑ Aerobic enzyme capacity
↓ Myoglobin	Shorter sarcomere
Damage/impaired regeneration	
Apoptosis	Oxidative stress
Autophagy	↑ Proteolysis / ↓ protein synthesis
Oxidative stress	Damage
↑ Proteolysis / ↓ protein synthesis	Apoptosis

Figure 4 Biological phenomena that have been observed in respiratory and limb muscles of COPD patients (clearly detrimental: black boxes; probably beneficial: white box). COPD, chronic obstructive pulmonary disease.

Comorbidities and aging

These are also potential additional contributors to the muscle dysfunction shown by COPD patients. On the one hand, many of the most frequent comorbidities of this respiratory disorder, such as chronic heart disease, diabetes and cancer also lead to muscle wasting and dysfunction (24,32,42,140,182). On the other hand, developed societies are characterized by an increased number of elderly individuals with chronic conditions such as COPD. Aging per se associates with loss of muscle mass (sarcopenia), fibrosis, mitochondrial efficiency, functional impairment in the neuromuscular junction (183-185), and progressive muscle inability to perform daily life tasks (186).

Many of the cellular and molecular events that occur in the muscles of COPD patients have been mentioned in the previous section when reviewing the etiopathogenic factors of muscle dysfunction. This is the case of local inflammation and oxidative stress, apoptosis, muscle injury, regeneration defects, imbalance between protein synthesis and destruction, loss of capacity of enzymes in the aerobic pathways, changes in fiber size and type proportions, and findings suggesting a myopathy. These, and other findings, will be discussed in more detail in the next section.

Biological phenomena observed in muscle (Figure 4)

Muscle inflammation

As previously mentioned, inflammatory phenomena have been observed in some of the muscles of COPD patients. Most authors have reported increases in the number of inflammatory cells in the peripheral muscles (100,187,188),

although not in the respiratory muscles of these patients (188). In contrast, there are major discrepancies among different authors with respect to the presence of inflammatory cytokines in skeletal muscles. Some of them have found increases of these substances in the patients' peripheral muscles (100), while others have reported exactly the opposite (98,189). As for respiratory muscles, there is only one available report, and this indicates that there is an increase in different proinflammatory cytokines in this population (44).

Oxidative and nitrosative stress within the muscle

This factor is important for both its action damaging DNA, proteins and cellular lipids, and its direct impact on muscle function. Increased levels of oxidative and/or nitrosative stress have been described both in respiratory and limb muscles of COPD patients (43,123,140,190-193). This appears to be the consequence of an increased production of ROS in mitochondria (193) as well as a decrease in local antioxidants (194), although the latter appears less affected in the particular case of respiratory muscles (195). Moreover, in COPD patients the increased baseline levels of oxidative stress seem to increase even more after intense exercise (169,175). Although oxidative stress can damage different cellular structures and modify key enzymes (140,175), its role in the increase of protein destruction and fiber atrophy is not very clear (190).

Muscle damage

Although not all the authors agree (196), different signs of damage have been reported in either peripheral or respiratory muscles of COPD patients. This evidence

has been observed both in the contractile structure of sarcomeres and also in the sarcolemma and other muscle structures (44,59,140,197). Interestingly, high intensity exercise appears to increase the level of muscle damage in these patients (59,198). However, as opposed to what happens in many myopathies, only small increments of fibrous and fat tissues have been observed in COPD muscles COPD (109). It should be emphasized that muscle damage is not necessarily a harmful phenomenon. As different animal models suggest, when its level is mild to moderate it can lead to muscle repair and adaptive remodeling (57,58).

Satellite cells and muscle regeneration program

These elements are closely related to the previous paragraph, since the maintenance of muscle structure depends on the balance between damage and repair/regeneration (*Figure 3*). Satellite cells are responsible for maintaining an adequate number of operational nuclei in muscle fibers, which in turn will favor an adequate protein synthesis and muscle mass. The number of satellite cells appears to be preserved both in the respiratory and the limb muscles of COPD patients (187,199). However, their regenerative capacity seems altered, at least in the latter, as suggested by the increase in internalized nuclei and decreased expression of late markers of regeneration (200). In keeping with this, cultured myoblasts (equivalent to satellite cells) obtained from COPD patients evidence problems in their later stages of differentiation, with difficulties in expressing adult myosins (200,201). This has been attributed to cell aging as suggested by telomere shortening (202).

Apoptosis

It must be clarified that the classical meaning of apoptosis and its histological signs is somewhat different in skeletal muscle (a syncytium) than in uninucleate cells. Indeed, here the death of one or more nuclei does not involve the death of the fiber since they can be replaced by those nuclei from satellite cells. Although the TUNEL technique has shown an increase of nuclei with 'apoptotic signs' in the muscles of COPD patients (either with preserved or reduced body weight) (188,203), true apoptosis has not been confirmed by electron microscopy (the gold standard technique) (188). Therefore, these signs are probably linked to the nuclear turnover, without the presence of real apoptosis.

Autophagy

This is a catabolic process involved in the elimination of excessive or altered cellular organelles. Signs of autophagy

have already been found in peripheral muscles of COPD patients, with and without weight loss (140,204).

Epigenetic alterations

These are changes in the expression of certain genes without any modification in the genome. Such changes have been reported both in respiratory and peripheral muscles of patients with COPD, either with preserved or reduced body weight (45,205,206), and can be considered as a response to either chronic overload or atrophic signals, respectively.

Muscle capillarization

Although some authors have reported a decreased capillary density in the lower limb muscles of patients (80,207,208), especially in those with early occurrence of fatigue during exercise (209), other researchers have failed to confirm this finding (86,210,211). Moreover, both the external intercostal and diaphragm muscles seem to show an increased capillary density (212,213), which would be added to the other aerobic adaptations observed in these muscles. It is possible that many of these changes in the number of blood vessels are related to the level of expression of the vascular endothelial growth factor (VEGF), which is decreased in limb muscles but increased in respiratory muscles of COPD patients (98,214). Finally, animal models have shown that either emphysema or hypoxia may result in an increase in the number and length of the capillaries, as well as on their contact surface with fibers (215,216).

Mitochondrial density and function

Again a discrepancy between limb and respiratory muscles has been observed for mitochondrial density. While their number is decreased or roughly preserved in the former (quadriceps and tibialis anterior muscles, respectively) (217), it appears to be increased in the diaphragm (51). There is also an important mitochondrial dysfunction in limb muscles expressed by an uncoupling between different steps of the respiratory chain, reduced aerobic enzyme capacity, increases in phenomena linked to apoptosis and increased production of free radicals (218).

Enzymes in aerobic and anaerobic pathways

The capacity of key enzymes involved in different metabolic pathways has also been studied in detail in muscles of COPD patients. It is possible, however, that in some cases the reported changes directly depend on modifications also observed in fiber phenotype and/or the number or efficiency of some cell organelles such as mitochondrias (32).

Respiratory muscles show activity increases in different enzymes participating in the oxidative pathways in COPD patients (196,219-221), while a decrease in enzyme capacity may occur in glycolytic pathways (222). On the contrary, lower limb muscles typically show less enzyme activity in their aerobic pathways, with maintenance or even increase in glycolytic enzymes (83,223,224). However, chronic respiratory failure appears to counterbalance some of these effects, since some oxidative enzymes increase their activity in those patients in such circumstances (225). Unfortunately, this would lead to metabolic uncoupling in these pathways, probably resulting in impaired muscle bioenergetics (78). Moreover, the loss of aerobic enzyme capacity can also be reverted by endurance training (131,224,226). Finally, the upper limb and shoulder muscles roughly seem to maintain the capacity of their key enzymes (94,221,222) and can even show increases in the enzyme activity within oxidative pathways in most severe COPD patients (94).

Changes in muscle fibers

This is one of the changes consistently observed in the muscles of COPD patients. Their limb muscles show a higher proportion of type II fibers (fast-twitch contraction, predominantly anaerobic metabolism) (86,190,227), while the diaphragm and intercostal muscles seem to show changes in the opposite direction since patients increase the proportion of type I fibers (slow-twitch contraction, aerobic metabolism and fatigue resistant) (48,61,228). All these changes depend on parallel modifications in the expression of adult myosin isoforms in response to those stimuli mentioned in preceding sections (with an apparent key role for the level of activity of each muscle) (48,49,229). It is worth noting that the proportion of slow-twitch fibers has recently been related to mortality in COPD patients (230). Furthermore, it seems clear that lower limb muscle fibers disclose a reduced size in those patients with loss of body weight (86,190,231). This atrophy is especially evident for type II fibers (190,231). As already mentioned, the situation is different in the muscles of the upper extremities, as they appear to keep their fiber cross sectional area (93,232). There is more discrepancy regarding the size of diaphragmatic fibers. Some authors have reported atrophy (233,234), while others have not been able to find such an abnormality in COPD patients (45,188,235). As regards to the function of the fibers, it seems to be altered in respiratory muscles (236,237) but surprisingly, not in limb muscles (238) of patients. In this regard, the diaphragm (237) as well as the intercostal muscle (236) show a decline in the strength of their fibers (normalized by size).

Protein synthesis and degradation

The imbalance between protein synthesis and breakdown appears as the key mechanism for the loss of muscle mass and function (42). Indeed, muscle mass is dynamically maintained by the balance between these two processes. When such a balance breaks and destruction prevails, muscle mass is reduced and that has an important impact on muscle function. It is well known that protein synthesis is reduced in underweight emphysema patients (239), but not in those with preserved body weight (240). Protein synthesis depends on the availability of substrates and the activity of signaling pathways. Regarding the former, several authors have reported that there is a reduction in plasma levels of glutamine, glutamate and alanine, as well as in some branched-chain amino acids (such as leucine) in COPD patients with low weight (239,241-243). The results for other amino acids are much more controversial (244). With regards to signaling pathways, the protein kinases B (Akt) and rapamycin (mTOR), which are activated in response to the input of nutrients and anabolic hormones, play a determinant role in muscle protein synthesis. Some authors have observed a decrease in the expression of Akt in limb muscles of COPD patients (204), while others have not confirmed these changes (206). Moreover, it has been reported that in COPD patients with severe hypoxemia, high intensity exercise reduces phosphorylation of Akt, potentially contributing to decreased protein synthesis (245). Among the factors that may explain the deficits in the synthesis of proteins in COPD patients with low body weight are the changes in anabolic hormones or their signaling pathways, and the presence of systemic inflammation and oxidative stress (246).

Protein breakdown in turn can occur through different pathways, including that of the proteasome. This pathway requires previous ubiquitination of the target protein, which needs the intervention of atrogenin-1 and MuRF1, both controlled by FoxO transcription factors and their regulators (247,248). These factors seem to be overexpressed in skeletal muscles of COPD patients (238,249,250). Moreover, protein ubiquitination has already been demonstrated in both limb muscles (140) and diaphragm (251) of such patients. In a second pathway, proteins can be degraded by the lysosomal enzyme system, which includes lipases, glycosidases and cathepsins, among others. This system is closely related with the aforementioned cell phenomenon of autophagy (252), which increases in the

muscles of COPD patients (204), although some authors have only been able to find it in those patients associating low weight (140). The third catabolic pathway is that of calpains, not lysosomal proteases that are highly dependent on calcium concentration. To date no studies have clarified the role of this proteolytic system in COPD patient muscles. Finally, there is also the pathway of caspases, closely linked to apoptosis. This can be activated by different factors, including exercise (253). Unfortunately, to date the results regarding its role in COPD are controversial. Some authors have found no changes in the levels of these enzymes in either peripheral or respiratory muscles of those patients with preserved body weight (188), while others have reported an increase in caspase 3 activity in the diaphragm of these patients (251). Moreover, the activity of caspases is still unknown in the muscles of patients with weight loss or following high intensity exercise. As with reduced protein synthesis, the activation of their degradation may be due to different factors present in COPD including tobacco smoking, exacerbations, inflammation, oxidative stress and treatment with steroids (246).

Signs of true myopathy

For a long time there was debate as to whether muscle abnormalities associated with COPD constitute a real myopathy (254). Strictly speaking, the definition of myopathy needs a number of specific muscle findings such as necrosis, inflammatory infiltrates, immune phenomena and/or inclusion bodies. Of all of them, only paracrystalline inclusions have been reported in the diaphragm of one isolated patient (60). Therefore, in recent years there is a strong tendency to consider that COPD muscle abnormalities do not constitute a true myopathy. The only exception is the myopathic alteration secondary to treatments with systemic steroids.

Conclusions

Taking everything into account, it can be stated that skeletal muscles show structural and functional changes in COPD patients, and that these changes are the result of the complex interaction of multiple factors, which is specific to each particular muscle. Tobacco, systemic inflammation and nutritional abnormalities seem to be important for all the different muscle groups, whereas geometrical changes occurring in the thorax are specifically harmful for respiratory muscles, and deconditioning is more detrimental for peripheral muscles.

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Oxygen therapy for COPD

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Abstract: Chronic obstructive pulmonary disease (COPD) is a leading cause of disability and death globally, characterised by progressive breathlessness, loss of function and, in its later stages, chronic hypoxaemia. Long-term continuous oxygen therapy increases life expectancy in patients with severe resting hypoxaemia. However, there are few data to support the use of oxygen in patients with only mild hypoxaemia and more research is required to determine any benefits of oxygen supplementation in COPD in such individuals.

Keywords: Oxygen; oxygen usage; chronic obstructive pulmonary disease (COPD)

Introduction

As we mark the centenary of the First World War, it is opportune to recall that the medical use of oxygen was popularised when Haldane first used it in treating gas inhalation injuries during World War I (1). Physicians before and after Haldane used oxygen intermittently for treatment of a range of conditions, but it was not until the 1950s (2) saw the development of techniques to facilitate point of care arterial blood gas analysis that the use of oxygen and its titration to a measured sample became common place. In the mid-late 20th century two randomised trials were performed almost simultaneously which have had a profound effect on the management of chronic obstructive pulmonary disease (COPD) over the last three decades.

Benefits of long-term oxygen therapy in COPD: review of original trials

In the 1970s physicians in the United Kingdom and the United States conducted two separate but similar studies to determine whether treating hypoxaemia in COPD could improve mortality. These trials, the UK Medical Research Council (MRC) (3) study and the US Nocturnal Oxygen Therapy Trial (NOTT) (4), showed that long-

term oxygen therapy when given for greater than 15 hours per day improved survival in patients with COPD and chronic hypoxaemia (PaO$_2$ ≤55-60 mmHg), with or without hypercapnia. The UK study included 87 hypercapnic patients with PaO$_2$ 40-55 mmHg on two measurements over a 3-week exacerbation-free observation period. Exclusions were co-existent fibrotic lung disease, pulmonary thromboembolism, hypertension and ischaemic heart disease or other life-threatening illness. In this unblinded controlled study, patients received oxygen via concentrator for 15 hours/day or no oxygen at all. No portable oxygen was provided and patients were not excluded if they continued to smoke. Patients were followed for three years or until death (3). The US Nocturnal Oxygen Treatment Trial (NOTT) enrolled 203 patients with stable hypoxaemia (PaO$_2$ ≤55 or 59 mmHg in the presence of cor-pulmonale, haematocrit ≥55% or electrocardiographic evidence of P pulmonale) on two measurements over a 3-week exacerbation free period (4). The patients received continuous or nocturnal oxygen and were also followed for a period of 3 years or until death. Those on continuous oxygen received portable oxygen as well. In this study the oxygen flow rate provided was sufficient to increase PaO$_2$ to 60-80 mmHg, with flow rates increased by 1 L/min during sleep and exercise. In both studies the majority of subjects

were male with a mean age of 65 years. In the MRC study mortality at three years was 45.2% in the oxygen treated group and 66.7% in controls. In the NOTT mortality rates at 24 months were 27% for the continuous group and 41% for the nocturnal group, demonstrating a significant survival advantage in the continuous oxygen group, in whom the average oxygen usage was 18 hours per day, compared with the nocturnal oxygen only group.

Because of the many similarities between these two trials, the results came to be considered together to demonstrate that oxygen for 15 hours per day was better than no oxygen (data from the MRC study) and that continuous oxygen had a greater mortality benefit than nocturnal oxygen (data from the NOTT) (3,4). These results significantly altered treatment of hypoxaemic COPD and, to this day, domiciliary oxygen is the only therapy (apart from smoking cessation) that has been shown to reduce mortality in COPD (3-5). As a consequence, most international guidelines for the management of oxygen therapy in COPD recommend that oxygen should be considered for patients with stable COPD, who have an oxygen partial pressure in arterial blood (PaO_2) consistently less than or equal to 55 mmHg (7.3 kPa) at rest when awake and breathing air and for patients with PaO_2 56-59 mmHg (7.4-7.8 kPa) with polycythaemia (haematocrit >0.55) or clinical, electrocardiographic or echocardiographic evidence of pulmonary hypertension and or right heart failure (6-9). At assessment, the patient's condition must be stable and all reversible factors (such as anaemia) should have been treated (9). In the Thoracic Society of Australia and New Zealand Position Statement, it is recommended that assessments should be made at least one month after the patient has stopped smoking, given that gas exchange may improve substantially on ceasing smoking (9,10). However, other guidelines do not necessarily recommend smoking cessation and it is to be remembered that at least one of the two studies on which the recommendations are based included current smokers (3). It is generally recommended that oxygen should be used for as many hours of the day as possible; ideally a minimum of 15+ hours.

These recommendations for oxygen therapy are based on two randomised non-placebo-controlled trials containing fewer than 300 patients, conducted over 30 years ago. The indications for prescription of oxygen therapy were the results of pragmatic decisions by the trial designers and the studies were performed in COPD populations that would not necessarily be representative of today's COPD patients, many of whom are older and have more

co-morbidity. There have not been any subsequent, high quality randomised controlled studies of long-term oxygen therapy in severely hypoxaemic COPD. A retrospective analysis of South Australian COPD patients prescribed long term home oxygen from a single centre between 1977 and 1999 found the annual death rate was 20-33% per year—worse than that for the control (no oxygen) group in the UK MRC study (11). Reasons for the differences between the prospective MRC study and this retrospective review may include the older age of the patients being prescribed oxygen, the presence of co-morbidities, continued smoking, inadequate treatment of hypoxaemia, lack of adherence or the fact that this was a real world situation rather than a clinical trial. It would seem important to clarify the true impact of long term oxygen therapy on all-cause mortality in those patients who are currently receiving it, many of whom are elderly with multiple co-morbidities. The introduction of local or national databases aimed at capturing information about patients receiving home oxygen could provide an ideal means of obtaining prospective data on patients currently receiving oxygen therapy. The introduction of such a national database in Denmark was associated with an improvement in adherence to guidelines and slight reduction in mortality (12).

Other benefits of long-term oxygen therapy

There is little convincing evidence from studies to date that long-term oxygen therapy has significant benefits other than on survival. Indeed, the mechanism for the improvement in survival with oxygen therapy still remains unclear, despite the observation of small improvements in some haemodynamic parameters in the NOTT (4). Endpoints in the MRC study were physiological characteristics and mortality (3). In the NOTT, neuropsychological tests were assessed in both continuous and nocturnal oxygen groups at baseline and at six months. Only 42% of patients showed improvements at six months and there were no differences between the continuous and nocturnal groups (13). It should be reiterated that the lack of a control group (intranasal air) makes it difficult to determine whether the improvements in neuropsychiatric function were due to more than placebo effect.

With the potential restriction of movement imposed by long-term continuous oxygen therapy, it is possible that the treatment may only prolong suffering rather than improving quality of life (QOL). Non-placebo-controlled trials differ in showing either no benefit or a small benefit

in health related QOL in subjects commenced on long term continuous oxygen therapy (14,15). Although small improvements in QOL were found in the NOTT (4), the study did not have a placebo (air) arm and thus the presence of a placebo effect is not excluded. Whether oxygen therapy is worthwhile in the context of a particular individual's management should be determined by a comprehensive clinical assessment rather than solely, or mainly, by the increase achieved in PaO_2.

Nocturnal desaturation in COPD: is oxygen therapy indicated?

The clinical consequences of nocturnal hypoxaemia in patients with COPD and daytime PaO_2 ≥60 mmHg (8.0 kPa) are unclear. Although it has been suggested that repetitive transient desaturations throughout sleep may be one mechanism underlying the development of pulmonary hypertension in COPD, the primacy of hypoxia as a driving force in the development of pulmonary hypertension in COPD is now questioned, with systemic inflammation suggested as one of several possible alternative factors. Chaouat et al. showed that elevated circulating levels of interleukin-6 correlated with elevations in mean pulmonary artery pressure (16).

C-reactive protein levels have also been shown to correlate with both pulmonary artery pressure and levels of endothelin-1, a potent vasoconstrictor postulated to play a role in vascular remodelling and pulmonary hypertension (17). The issue of the role of inflammation in pulmonary hypertension complicating COPD remains controversial (18). However, in vitro animal models of pulmonary artery remodelling related to tobacco smoke studies support such a mechanism (19).

Although a New Zealand study suggested that isolated nocturnal desaturation was very uncommon in a general COPD outpatient population, and that patients with nocturnal desaturation had no worse sleep quality, QOL or daytime somnolence than those without desaturation (20), other studies have reported sleep fragmentation (21) as well as surges in both systemic and pulmonary blood pressure (22) as a consequence of nocturnal desaturation. Fletcher et al. suggested that nocturnal desaturation in patients with PaO_2 >60 mmHg occurred in 27% of patients, where desaturation was defined as a desaturation below 90% for five minutes or more, with a nadir saturation of at least 85% (23). Although a small retrospective study by Fletcher et al. suggested that patients with nocturnal oxygen desaturation had a poorer survival than those without (24) a subsequent small prospective study by Chaouat et al. found that COPD patients with nocturnal desaturation did not develop pulmonary hypertension more than a group of patients without nocturnal desaturation and their prognosis was no different over 6 years of follow-up (25). Whether nocturnal hypoxaemia alone can lead to substantial pulmonary hypertension remains controversial. Fletcher et al. demonstrated that nocturnal supplemental oxygen at 3 L/min over three years was associated with a smaller rise in pulmonary artery pressure than in a control group receiving supplemental air (26). However, there was no effect on mortality. A larger 2-year study of patients with COPD and modest daytime hypoxaemia [PaO_2 56-59 mmHg (7.4-7.8 kPa)] who desaturated to a pulse oximeter oxygen saturation (SpO_2) <90% for >30% of the night found no survival benefit in the group receiving oxygen supplementation and no effect on pulmonary haemodynamics (27). There is no international consensus regarding provision of nocturnal oxygen in COPD for patients with daytime PO_2 >60 mmHg.

Episodes of nocturnal hypoxaemia due to hypoventilation or worsening ventilation-perfusion in patients with COPD should be distinguished from those associated with sleep apnoea caused by upper airway obstruction, obesity hypoventilation syndrome or central sleep apnoea. Apnoea syndromes are diagnosed by overnight polysomnography and generally require other forms of therapy (such as continuous positive airway pressure or nocturnal ventilation) rather than supplemental oxygen.

Ambulatory oxygen

In those fulfilling criteria for long term continuous oxygen therapy

Ambulatory oxygen therapy may be used as part of continuous oxygen therapy, in which case its use is aimed at maximising the number of hours per day a person with COPD can use their oxygen for, at the same time as maintaining adequate physical activity including engaging in pulmonary rehabilitation. Pulmonary rehabilitation has been demonstrated to improve exercise capacity and QOL in COPD (28), whilst reduced physical activity is associated with increased risk for hospitalisation and for mortality (29). Patients in the "continuous" arm of NOTT, whose survival benefit was greatest, used both stationary and ambulatory systems in order to enable an average usage of 18 hours per

day (4). Extrapolation from the studies of long term oxygen therapy (3,4) may suggest that using ambulatory oxygen during activity would enhance the benefits of LTOT but there are few data to support this.

In those not fulfilling criteria for long term continuous oxygen therapy

Despite the observation of small, acute benefits of oxygen therapy during laboratory-based exercise tests in COPD (30), the subject of ambulatory oxygen use in patients who do not fulfil criteria for LTOT remains controversial. Ambulatory oxygen is often provided for patients who desaturate with exertion because of such short-term in-laboratory studies which have demonstrated modest improvements in exercise capacity and/or dyspnoea. Interestingly, these small benefits may be noted in patients who do not desaturate on exertion as well as in those who do and have been attributed to reductions in dynamic hyperinflation induced by a hyperoxia-driven reduction in ventilation (31,32). The underlying mechanisms for dyspnoea and exercise limitation in COPD are complex (33). In a study of ten patients with severe COPD and mild hypoxaemia by Somfay *et al.* (34), endurance time on a symptom-limited incremental exercise test was increased whilst breathing increasing concentrations of oxygen up to 50%, with small but statistically significant decreases in dyspnoea score, end-expiratory and end-inspiratory lung volume, minute ventilation and breathing frequency. These authors determined that there was a dose-dependent improvement in exercise endurance and dyspnoea which may be partly related to a reduction in hyperinflation and reduced breathing frequency. Although widely prescribed, usually on the basis of relief of exertional desaturation during a laboratory-based test, the use of domiciliary ambulatory oxygen is not strongly evidence-based. Two studies of cross-over design which examined the impact of portable oxygen therapy on QOL had conflicting findings in terms of QOL and a small multiple n-of-one study found no benefit (35-37). In an adequately powered study of patients with COPD who remained breathless on exertion despite maximal treatment, Moore *et al.* randomised patients without severe resting hypoxaemia to use ambulatory oxygen or ambulatory air at 6 L/min during exertion for 3 months at home (38). Included patients (n=143) had PaO_2 greater than 60 mmHg at rest on room air, with a third desaturating to SpO_2 <88% on exertion. Although there was a trend to improvement in both arms of treatment, there was no difference between supplemental

air and supplemental oxygen used during exercise with regards to dyspnoea, QOL or function, and the presence of exertional desaturation was not predictive of outcome. These results suggest a substantial placebo effect from the administration of intranasal gas, possibly relating to the wearing of nasal cannulae (39). Nonetheless, in the study by Nonoyama *et al.*, where investigators performed multiple n-of-1 studies, occasional patients (n=2 out of 27) achieved clinically significant reductions in dyspnoea, so blinded assessments may be useful in selected individuals (37).

Oxygen for pulmonary rehabilitation in COPD

Theoretical reasons to support the use of supplemental oxygen during training include the potential for amelioration of exercise-induced elevations in pulmonary arterial pressure (40,41) and the potential for reductions in minute ventilation and dynamic hyperinflation (34). However, there is no evidence to support this practice.

"Palliative" oxygen

Supplemental oxygen has been used in an attempt to provide symptomatic relief for patients with intractable dyspnoea due to terminal illnesses, including late-stage lung disease such as COPD, even in the absence of hypoxaemia. In the first large, international multi-centre trial examining this question, Abernethy *et al.* randomly allocated 239 patients with life-limiting disease, including COPD (64%) and cancer (16%), to receive oxygen or medical air (42). Both were delivered at 2 L/min through nasal cannulae via a concentrator for seven days. Primary outcomes were impact on breathlessness and QOL. The study found that both medical gases induced small improvements in dyspnoea and QOL, with more severe baseline breathlessness predicting this benefit. They also found that most of the improvement occurred in the first three days. The conclusion was that palliative oxygen had no benefits over medical air for relieving dyspnoea or for improving QOL for the whole population and that there were small improvements with both arms of the treatment. A therapeutic trial of medical air or oxygen over 3 to 4 days was thus proposed as a way of assessing those dyspnoeic patients who might benefit. It is thought that relief of breathlessness with either oxygen or air, as described in the abovementioned study, could be due to stimulation of nasal receptors by gas flow, however, the mechanism by which this occurs is not known. The role of air flow as an intervention has been explored and cold air directed on the face has been shown to

reduce breathlessness induced by inspiratory resistive loading and hypercapnia in normal subjects (39) and in patients with COPD (43). A placebo controlled study using a handheld fan in patients with intractable dyspnoea from different causes showed benefit (44).

Oxygen in moderate hypoxaemia

Despite the mortality benefits from long term oxygen therapy in patients with COPD and severe hypoxaemia, there is no such benefit for continuous oxygen supplementation in patients with milder degrees of hypoxaemia. A trial reported by Górecka and colleagues (45) found that LTOT (oxygen for a mean of 17 hours/day to raise PaO_2 to ≥65 mmHg) had no effect on survival over a mean observation period of 40.9 months in patients with COPD and only moderate hypoxaemia (56-65 mmHg). One hundred and thirty five patients with mean FEV_1 of 0.83 L were included. The overall mortality in this study was 11-12% per annum, which is close to that of patients on continuous oxygen therapy in the NOTT (4). Younger age, better spirometric values and higher body mass index predicted better survival.

Cognitive function, hypoxaemia and driving

Cognitive dysfunction has been described in people with COPD. The frequency of cognitive dysfunction varies depending upon the battery of neuropsychological tests used, with the domains most influenced being memory and attention. COPD diagnosis was linked with an 83% higher risk for developing non-amnestic mental decline in a recent prospective study of aging adults (46). Although hypoxaemia may be one several mechanisms by which COPD induces mild cognitive impairment in COPD there is limited evidence for benefit of long term oxygen therapy on cognition (47). Previous guidelines on fitness to drive in Australia recommended patients on long term oxygen therapy should use supplemental oxygen whilst driving a motor vehicle (48). However, there is no evidence for either improvement in cognition (49,50), or in simulated driving performance with acute oxygen therapy in this patient group (51). Thus, there is currently no recommendation for hypoxaemic patients to use portable oxygen therapy whilst driving in Australia and/or New Zealand (52).

Risks of oxygen in acute exacerbations of COPD

It has been known for decades that patients with

acute exacerbations of COPD may develop worsening hypercapnia with the application of supplemental oxygen, particularly at high concentrations. The mechanisms underlying this phenomenon relate to a combination of (I) worsening ventilation perfusion mismatch secondary to attenuation of hypoxic pulmonary vasoconstriction; (II) the Haldane effect which involves displacement of carbon dioxide bound to haemoglobin by increased oxygen concentration; and (III) hypoventilation. The adverse effects of high concentrations of supplemental oxygen were recently confirmed in a study by Austin *et al.* which reported an increase in mortality for patients randomised to receive high concentration oxygen versus those who received low concentration titrated oxygen to a target saturation range of 88-92% when transported via ambulance with acute exacerbation of COPD (53).

Current research and future research needs

Oxygen is a widely used treatment for COPD and a range of other chronic lung diseases. Apart from the demonstrated evidence of mortality benefit with LTOT in patients with severely hypoxaemic COPD and minimal co-morbidities, the evidence for significant benefit with oxygen in a range of other circumstances is lacking.

Currently recruiting studies

Long term oxygen treatment trial (LOTT)
The currently recruiting LOTT is sponsored by the National Heart Lung and Blood Institute and Centers for Medicare & Medicaid Services in the United States. Its stated aims are to determine whether continuous supplemental oxygen increases time to a composite outcome of all-cause mortality or all-cause hospitalisation as well as examining deterioration in QOL. Patients will receive either continuous oxygen for 24 hours/day if they have moderate resting hypoxaemia or supplemental oxygen for sleep and activity for those with exercise desaturation. There will be no placebo (supplemental air) arm which is disappointing given there is clinical equipoise. The reason for the absence of a placebo arm is unclear, but it may relate to the (no doubt significant) costs of supporting such a study. The absence of a placebo arm is extremely disappointing as there are clearly demonstrated placebo effects of intranasal gas flow and such a study would have provided an excellent opportunity to explore the question in detail. The

NOTT was not able to clearly determine any benefits on QOL or cognition because of the lack of a placebo arm.

Supplemental oxygen in pulmonary rehabilitation trial (SuppORT)

This currently recruiting trial sponsored by the Australian National Health and Medical Research Council (NHMRC) is a randomised controlled trial of supplemental oxygen versus medical air in people with COPD aimed at determining whether supplemental oxygen improves the exercise capacity and QOL of patients with COPD who desaturate with exertion.

Studies of oxygen therapy are difficult to undertake because of the severely disabled population of patients involved and the lack of funding sources, however, further studies are needed. Research questions include whether oxygen for exertional use can improve QOL and activity levels if the delivery device is more "user friendly". Although recent studies of exertional oxygen used over medium term durations in COPD have not demonstrated benefits over portable air; it is the case that patients do not use their portable oxygen delivery devices more that about 40 minutes per day. Future studies should try and determine whether the absence of oxygen use relates to the ineffectiveness of the treatment or to the physical properties of the portable device being clumsy or heavy or embarrassing to use and cancelling out any the small magnitude of any potential benefits gained.

A large study to determine whether nocturnal desaturation has short and long term sequelae on sleep quality, pulmonary haemodynamics and QOL is also warranted.

Conclusions

Oxygen therapy is known to improve mortality in patients with severe hypoxaemia and COPD. Patients currently receiving this treatment are often older and have more co-morbidities than the patients who were enrolled in the original long term oxygen studies. Further studies and the development of national and perhaps international registries should allow clarification of the impact of oxygen therapy on COPD patients receiving oxygen therapy currently. Benefits from oxygen in patients with milder degrees of hypoxaemia who may desaturate on exertion or nocturnally are unclear and require further study. Such future prospective studies should include a placebo arm in order to distinguish benefit due to oxygen from placebo effect.

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Pulmonary rehabilitation for COPD: are programs with minimal exercise equipment effective?

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Abstract: Pulmonary rehabilitation is an essential component of chronic obstructive pulmonary disease (COPD) management with strong evidence supporting the efficacy of pulmonary rehabilitation to improve exercise capacity and quality of life, as well as reduce hospital admissions. However, it is estimated that only 2-5% of people with COPD who could benefit from pulmonary rehabilitation have access to programs. Most research on the benefits of pulmonary rehabilitation has used equipment such as cycle ergometers and treadmills for endurance training and weight machines for resistance training. To enable greater availability of pulmonary rehabilitation, the efficacy of exercise training using minimal equipment needs to be evaluated. Randomised controlled trials that used minimal, low cost equipment for endurance (eight trials) and strength training (three trials) compared to no training in people with COPD were evaluated. Statistically and clinically significant differences in functional exercise capacity and quality of life, as well as improvements in strength were demonstrated when exercise training with minimal equipment was compared to no training [six-minute walk test: mean difference 40 (95% CI: 13 to 67) metres; St George's Respiratory Questionnaire: mean difference -7 (95% CI: -12 to -3) points]. While the number of studies is relatively small and of variable quality, there is growing evidence that exercise training using minimal, low cost equipment may be an alternative to equipment-intensive pulmonary rehabilitation programs.

Keywords: Chronic obstructive pulmonary disease (COPD); pulmonary rehabilitation; exercise training; walking training; elastic resistance bands

Introduction

Pulmonary rehabilitation is an essential component of chronic obstructive pulmonary disease (COPD) management with strong evidence supporting its efficacy (1). Clinically significant improvements in exercise capacity, symptoms of dyspnoea and fatigue, quality of life and reductions in hospital readmission have been documented in Cochrane reviews of randomized controlled trials of pulmonary rehabilitation (1,2).

Recent guidelines (3,4) and statements (5) from major respiratory organisations have outlined the key elements of pulmonary rehabilitation which include supervised exercise training programs of at least 2-3 sessions per week for 6-8 weeks in duration. Guidelines recommend that the exercise session include endurance exercise training and resistance training (3,4).

Endurance training

Physiological studies have demonstrated that lower limb endurance training in people with COPD can decrease ventilatory demand at a given level of exercise due to changes at the muscle level that include increased muscle fibre capillarisation (6,7), mitochondrial density and muscle oxidative capacity (8-10). Such training adaptations improve the aerobic capacity of the muscle, delaying the

onset of lactic acidosis and, as such, reduce the ventilatory requirements for exercise at equivalent pre-training work rates (11). This is reflected in the ability of people with COPD to perform equivalent work rates for longer after training as well as achieving higher peak work rates (11).

In addition to lower limb endurance training, endurance training of the upper limb using either supported arm exercise (arm cranking) or unsupported arm exercise (free weights) has been shown to reduce ventilatory demand and improve arm exercise capacity (12-14). Such improvements are task specific (15), therefore exercise mimicking daily activities may be of greater functional relevance for people with COPD (16,17).

Resistance training

Reductions in skeletal muscle strength are evident in people with COPD (18,19) and may affect the ability to perform functional activities. Resistance training improves strength in people with COPD (20-22). Importantly, gains in strength may improve the performance of functional tasks, such as stair climbing and standing from a chair (21). Resistance training may also improve endurance capacity. Significant improvements in cycle endurance capacity were demonstrated in studies comparing strength training with no intervention (20,22). However, the gains in endurance capacity from resistance training were small compared to those that could be elicited from endurance training (20,22).

Besides improved exercise capacity, pulmonary rehabilitation programs have been shown to significantly improve health-related quality of life (1). It should be noted that while exercise training is considered the key component to achieve changes in exercise capacity and quality of life, exercise training often occurs as part of a comprehensive pulmonary rehabilitation program which includes education, anxiety and dyspnoea management, smoking cessation support, and nutritional advice. These additional components of pulmonary rehabilitation may enhance the outcomes of an exercise training regimen. However, a recent large randomised controlled trial of the addition of education to an exercise training program compared to exercise training alone showed no between group differences in exercise capacity, quality of life, physician visits, medication use or hospital admissions (23).

Access to pulmonary rehabilitation

Despite the high level evidence of the effectiveness of pulmonary rehabilitation, access to pulmonary rehabilitation programs worldwide is low. While reliable data on access to pulmonary rehabilitation programs for people with symptomatic COPD is not easily available, a number of studies have estimated that only 2-5% of people with COPD who could benefit from pulmonary rehabilitation have access to programs (24-26).

Most studies of the effectiveness of pulmonary rehabilitation have been performed in large metropolitan centres with well-equipped gymnasiums. There is a need to evaluate whether low cost programs with minimal equipment can achieve similar benefits. If programs providing pulmonary rehabilitation with minimal equipment are shown to be effective, the availability of pulmonary rehabilitation may be improved.

Exercise programs using minimal equipment

There have been a number of randomised controlled trials of endurance exercise training compared to standard care (no exercise training) in people with COPD in which the training mode required only minimal equipment [for example walking exercise (27-33), sit-to-stand (34), stepping (28,34)]. These trials were identified either from the most recent Cochrane review of pulmonary rehabilitation (1) or new trials published (in English) since that review based on a systematic search of Medline and Physiotherapy Evidence Database (PEDro) databases to identify randomised controlled trials of pulmonary rehabilitation where low resources were used. *Table 1* provides a description of the interventions in these trials.

The methodological quality of the trials was determined by the PEDro score (35). The PEDro score is a valid (36) and reliable (37) measure of the methodological quality of a clinical trial. The PEDro score out of ten is based on a criterion that considers the internal validity of the trial and whether the trial has adequate statistical data to make it interpretable (35). If there was sufficient data for common outcome measures from a number of trials, a meta-analysis was performed. For any meta-analysis, the weighted mean differences (WMD) were determined from the difference between the pre- and post-intervention changes in the intervention and control groups. If change scores had not been presented in the study they were determined by subtracting the post-intervention means from the baseline means. If the standard deviation of change scores was missing, the standard deviations (SD) of the baseline and post-intervention means were pooled according to the equation: SD of mean change scores $=\sqrt{((SD^2 post + SD^2 baseline)/2)}$ (38).

Table 1 Randomised-controlled trials using minimal equipment for endurance training

Study [year]	PEDro score/10	Participants (n) intervention/control	Intervention	Outcome measures
Hernández [2000] (27)	4	20/17	M: walking; I: 70% ISWT speed; D: 1 hour; F: 6 days/week; L: 12 weeks; S: every 2 weeks	ICT; ISWT; CRQ; MRC Dyspnoea
Ringbaek [2000] (28)	3	17/19	M: Walk/Jogging, stair climbing, resistance; I: dyspnoea 4-5; D: 1 hour; F: 2×/week; L: 8 weeks; S: 2×/week (physio); Other: Home training with elastic resistance bands	6MWT; SGRQ
Singh [2003] (30)	4	20/20	M: Walking; I: 'sub-maximal speed'; D: 30 minutes; F: 2×/day; L: 4 weeks; S: 1×/week at home; Other: ACT and pursed lip breathing	6MWT; CRQ
Murphy [2005] (34)	4	13/13	M: stepping, sit-to-stand; upper limb elastic band resistance; I: dyspnoea 3-5; D: 30-40 min; F: 2×/week; L: 6 weeks; S: 2×/week; Other: exercise 15 min on other days	ISWT; 3 min step test; Strength: knee ext & hand grip; MRC dyspnoea; SGRQ
Boxall [2005] (33)	5	23/23	M: walking; I: based on 6MWT dyspnoea and desaturation; D: 2-30 min; F: daily; L: 12 weeks; S: 1×/week for 6 weeks; then 1× every 2 weeks for 6 weeks; Other: arm exercises	6MWT; SGRQ, Hospital admissions
Breyer [2010] (29)	5	30/30	M: outdoor nordic walking; I: 75% HRmax; D: 1 hour; F: 3×/week; L: 12 weeks; S: 3×/week; Other: education 1×/week	Physical activity (triaxial accelerometer); 6MWT; SF-36; HADS
Ho [2012] (31)	7	19/20	M: paced walking to music; I: 80% VO_2peak from ISWT; D: 30 minutes; F: 5×/week; L: 12 weeks; S: 1×/month when tempo adjusted; Other: control group 'usual exercise' 5×/wk. Daily exercise diary for both groups.	ISWT; SGRQ; Hospital admissions
Casey [2013] (32)	7	143/134	M: walk; I: dyspnoea or fatigue 3-4; D: 20 minutes; F: 2-3×/week; L: 8 weeks; S: weekly diary review; Other: arm and leg exercises; weekly education	CRQ; ISWT

Intervention, intervention group; Control, control Group; M, mode; I, intensity; D, duration; F, frequency; L, length; S, supervised; 6MWT, six-minute walk test; ISWT, incremental shuttle walk test; ICT, incremental cycle test; CRQ, Chronic Respiratory Disease Questionnaire; SGRQ, St George's Respiratory Questionnaire; HADS, Hospital Anxiety and Depression Scale; ×/, times per; MRC, Medical Research Council; SF-36, Short-Form 36 quality of life questionnaire; ACT, airway clearance techniques; HR, heart rate; VO_2, oxygen consumption.
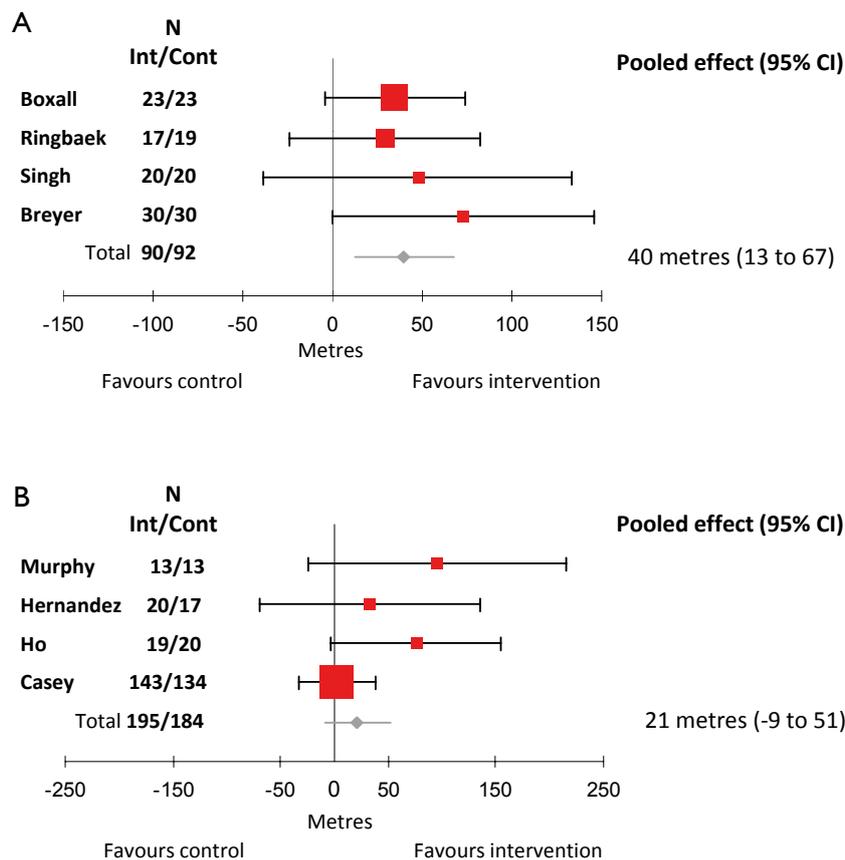
Figure 1 Mean difference (95% confidence interval) for the effect of exercise training using minimal equipment (intervention) compare to no exercise (control) by pooling data from (A) four studies (n=182) that used the six-minute walk test as an outcome and (B) four studies (n=379) that used the incremental shuttle walk test as an outcome. N, number; Int, intervention; Cont, control.

Exercise capacity

The studies of supervised lower limb endurance training in which minimal equipment has been used have mostly prescribed walking training (27-33). Four of these studies used the six-minute walk test (6MWT) to evaluate whether these training modes improved functional exercise capacity (28-30,33).

For the 6MWT, a meta-analysis included 90 participants in the exercise training group and 92 in the control group and showed a mean difference of 40 (95% CI: 13 to 67) metres in favour of the exercise group (*Figure 1A*). This difference in 6MWT distance was greater than the minimal important difference (MID) for the 6MWT which has been reported as 25 (95% CI: 20 to 61) metres (39). These combined studies suggest that walking training is adequate to improve functional exercise capacity.

Four studies used the incremental shuttle walk test as a measure of change in peak exercise capacity (27,31,32,34). For the ISWT, a meta-analysis included 195 participants in the exercise training group and 194 in the control group and showed no significant difference between the exercise group and the control group [mean difference 21 (95% CI: –9 to 51) metres] (*Figure 1B*). The ISWT is a measure of peak exercise capacity and an improvement requires the ability to walk faster. It may be that people with COPD who trained with minimal equipment can walk for longer after exercise training but may not be able to increase walking speed. None of these studies used the endurance shuttle walk test (40) as an outcome measure, which has been shown to be more sensitive to change following pulmonary rehabilitation (41).

The physiological changes induced by walking training have not been well studied. One study of predominately high intensity walking training in 25 participants with COPD demonstrated a significant reduction in lactate and

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ventilation at isotime on an incremental cycle test after walking training (42). These data provide some evidence of physiological responses in skeletal muscle due to walking training. In contrast, 20 participants in an eight-week, self-monitored, 5 days/week, home walking training program showed no reduction in lactate or ventilation on a constant work rate treadmill test after training (43). Randomised controlled trials with larger sample sizes are required to demonstrate whether physiological changes during a walking test are elicited after walking training.

Quality of life

Quality of life is an important outcome measure for pulmonary rehabilitation. The most commonly used health-related quality of life questionnaires in the included studies were the Chronic Respiratory Disease Questionnaire (CRQ) (44) and the St George's Respiratory Questionnaire (SGRQ) (45). Three studies reported the dyspnoea and fatigue domains of CRQ (27,30,32). A meta-analysis of these studies which included 183 participants in the exercise group and 171 in the control group showed a significant difference in dyspnoea of 0.48 (95% CI: 0.2 to 0.7) points (*Figure 2A*) and fatigue of 0.42 (0.2 to 0.7) points (*Figure 2B*) in favour of the exercise group. While these improvements were statistically significant, the MID for dyspnoea and fatigue is 0.5 points (46). Therefore, it could be considered that exercise training with minimal equipment resulted in a borderline clinically important improvement in the dyspnoea domain of the CRQ, however did not quite reach the MID for the fatigue domain. These mean differences are also less than those reported for studies that included training with exercise equipment (1).

The SGRQ gives a total score for quality of life and four of the included studies used this as an outcome measure (28,31,33,34). A meta-analysis which included 72 participants in the exercise group and 75 in the control group showed a mean difference in quality of life of –7 (95% CI: –12 to –3) points in favour of the exercise group (*Figure 2C*). A lower score in the SGRQ indicates better quality of life and the MID for SGRQ total score is –4 points (47), indicating that exercise training with minimal equipment is adequate to achieve clinically relevant improvements in health-related quality of life.

Prescribing walking training

A number of the studies in *Table 1* prescribed walking

intensity based on symptoms of dyspnoea (28,32,33). However, intensity of walking training can be prescribed from the initial field walking tests of either the 6MWT or the ISWT. Walking at 80% of the average 6MWT speed has been shown to elicit a mean (± SD) oxygen uptake (VO_2) of 77% (±13) of VO_2peak (48), whereas walking at 70% peak ISWT speed has been has been shown to elicit an oxygen uptake (VO_2) of 76% (±11) of VO_2peak (49). Exercise training above 50% VO_2peak is recommended, as exercise above this intensity is usually sufficient to achieve physiological training effects (50), with higher intensities possibly achieving greater training responses (11,51).

Resistance training

Three randomised controlled trials were identified that used minimal equipment for resistance training (52-54). One study compared a 12-week program of once a week supervised and twice a week home-based resistance training exercises such as sit-to-stand, seated row, lunges, simulated lifting, chest press using elasticised resistance bands, compared to no training (53). The trial quality was a PEDro score of 7. Results showed a small mean difference in knee extensor strength in favour of the exercise group with no differences in other outcomes such as 6MWT or health-related quality of life. More recently a high quality randomised controlled trial (PEDro score 8) of supervised elastic band resistance training plus patient education three times per week for eight weeks compared to patient education alone reported significant mean differences in 6MWT, unsupported arm exercise, and muscle strength in favour of the exercise group (52). Interestingly, a study that compared elastic resistance band training to conventional equipment-based resistance training demonstrated that both groups improved strength with no differences between groups (PEDro score 6) (54). Although these studies only equate to limited evidence, the findings suggest that supervised training with resistance bands may be an appropriate substitute for equipment-based resistance training in people with COPD.

Other less conventional training modes using minimal equipment

Tai Chi

Tai Chi is an ancient Chinese martial art which incorporates elements of strengthening, balance, postural alignment and concentration. It represents a mode of training which does
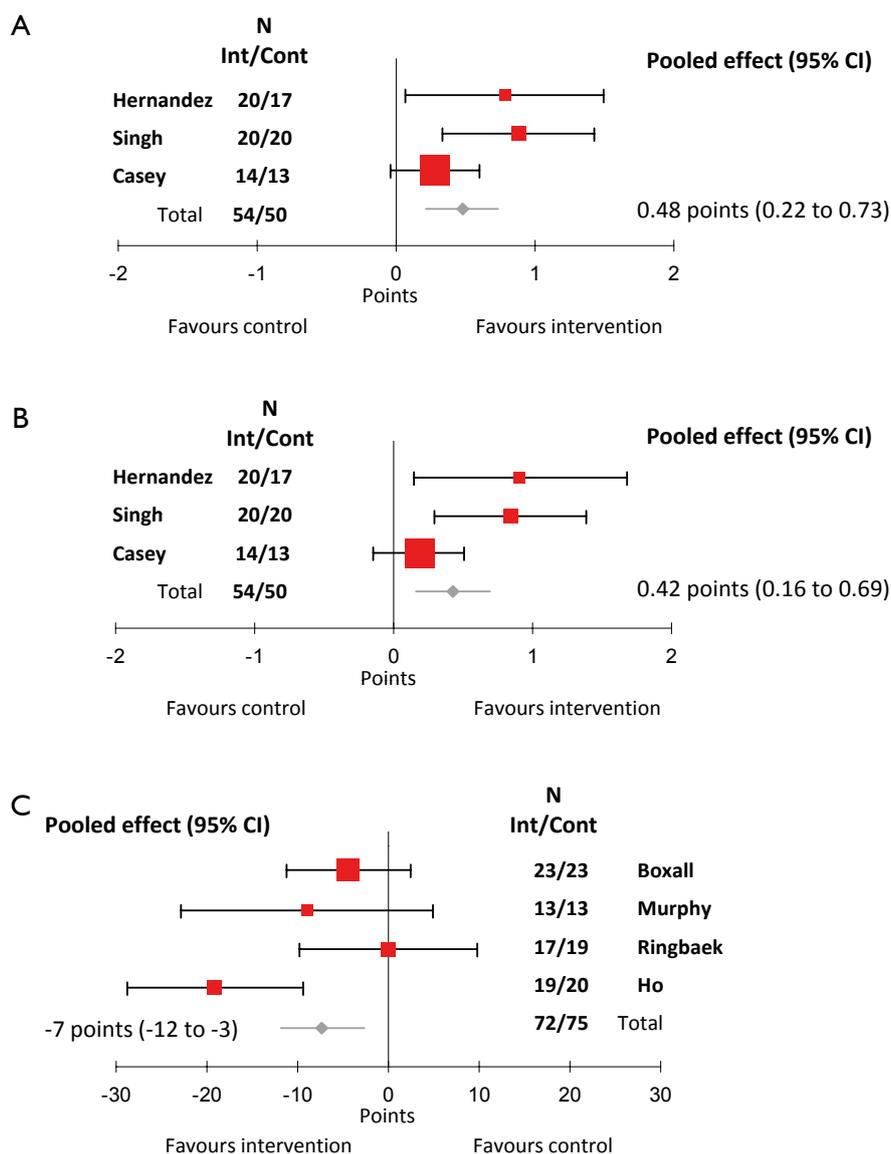
Figure 2 Mean difference (95% confidence interval) for the effect of exercise training using minimal equipment (intervention) compare to no exercise (control) by pooling data from (A) three studies (n=354) that used Chronic Respiratory Disease Questionnaire (CRQ) Dyspnoea as an outcome; (B) three studies (n=354) that used CRQ Fatigue as an outcome and (C) four studies (n=147) that used St George's Respiratory Questionnaire Total Score as an outcome. N, number; Int, intervention; Cont, control.

not require a specific training venue, can be performed without exercise equipment, and promotes aerobic, strength and balance training simultaneously. While there has been a recent systematic review of Tai Chi in COPD the included studies were of low methodological quality (55). Recently, a randomised controlled trial of Tai Chi in COPD with a high methodological quality (Pedro score 8) (56) demonstrated that Tai Chi training resulted in a significant difference in endurance shuttle walk test (ESWT) time

and SGRQ Total score in favour of the Tai Chi group compared to the control group (no exercise training) [ESWT mean difference 384 (95% CI: 186 to 510) seconds; SGRQ Total score –11 (95% CI: –18 to –4) points]. These between group differences exceeded the minimum clinically important differences for these outcomes. Balance and quadriceps strength were also significantly increased in the Tai Chi group compared to the control group. Importantly, Tai Chi elicited a VO_2 of approximately 63% VO_2peak

which would be adequate to achieve physiological training effects (54). This study provides some support for Tai Chi as a mode of training to improve exercise capacity and health-related quality of life in COPD.

Further research is needed with larger randomised controlled trials comparing pulmonary rehabilitation programs using minimal equipment with programs using gymnasium equipment. Such studies will help to determine the effects of minimal equipment programs benchmarked against the standard programs. In addition, the effects of minimal equipment on the longer term maintenance of benefits should be evaluated. It is possible that programs using minimal equipment may transfer to the home environment more easily and promote continued exercise and hence maintenance of benefits.

Conclusions

The demand for pulmonary rehabilitation and the lack of available programs requires focus on alternatives to conventional equipment-based exercise training that can be more widely offered. There is growing evidence that exercise training using minimal equipment is effective in improving outcomes of functional exercise capacity and health-related quality of life in people with COPD.

Acknowledgements

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Role of indacaterol, a once-daily bronchodilator, in chronic obstructive pulmonary disease

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Abstract: Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction that can lead to lung destruction and dyspnea. Although there has been a slight reduction in mortality in recent decades, COPD is still a serious health problem that has enormous costs and utilizes significant medical resources. There have been a number of pharmacologic interventions that have been developed for the treatment of COPD. Current guidelines recommend the use of long-acting bronchodilators for the treatment of moderate and severe stage COPD, since they have been shown to improve lung function, respiratory symptoms, and quality of life. Indacaterol is a once-daily beta2-agonist (β2-agonist) delivered by a single-dose dry powder inhaler used for the treatment of COPD. It is currently approved at a dose of 75 μg in the United States and a dose of 150 μg with a maximal dose of 300 μg in Europe and other countries. Several studies show that indacaterol was statistically superior to both long-acting β2-agonist, formoterol and salmeterol, as well as, noninferior to tiotropium. Indacaterol is generally well tolerated and has a good safety profile. Other studies show that there is an additive bronchodilator response with the addition of indacaterol to tiotropium, which would provide a once-daily treatment option for patient with moderate to severe COPD. This review discusses the pharmacokinetic, comparative efficacy and safety data for indacaterol.

Keywords: Indacaterol; chronic obstructive pulmonary disease (COPD); long-acting beta2-agonist (β2-agonist); tiotropium; salmeterol; formoterol; bronchodilator

Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung characterized by progressive airflow obstruction that is not fully reversible (1). Despite the improvements in medication and public education, COPD is still a serious health problem in the United States and around the world (2,3). In 2008, chronic lower respiratory diseases, which are primarily due to COPD, were the 3rd leading cause of mortality. It is estimated that the direct economic cost of COPD and asthma in 2008 is approximately $53.7 billion (3). Prior to 2000, studies show there was an increased rate of hospitalization and an age-adjusted mortality rate for COPD. Since 1999, there appears to have been a small decrease in age-adjusted mortality in men, but not in women. This reduction in mortality in COPD appears to correlate with the decreased prevalence in smoking, which has been steadily declining since 1965 (3). In 2010, the prevalence of smoking, the dominant risk factor for COPD, was 21.5% in men and 17.3% in women. In 2011, an estimated 6.5% of U.S. adults (approximately 13.7 million) were diagnosed with COPD. Between 1999-2011, the prevalence appeared to decline. Since much of this data is based on self-reporting, the true prevalence of COPD, based on spirometry, and the actual mortality rate are likely

underestimated (3).

Lung damage from COPD has several causes including proinflammatory mediators, oxidative stress, and proteolytic digestion of the lung tissue (1). The repetitive damage of the lungs leads to a slow and gradual progression of obstruction to airflow. Although initially asymptomatic, the continued destruction of airway and lung parenchyma with subsequent worsening airflow obstruction leads to the development of progressive symptoms of cough, dyspnea, wheezing and chest tightness (3). While there is clinical variability, most patients have a progression of disease severity leading to an acceleration in sensation of dyspnea, decrease in physical activity and social functioning which correlates with a decline in forced expiratory volume in 1s (FEV1) (3). Acute exacerbations of COPD can cause a rapid decline in lung function and increased dyspnea leading to poor quality of life, increased hospitalization and mortality (4). A number of pharmacologic interventions have been developed to improve lung function, as well as, decrease dyspnea and exacerbation rates (5). Long and short acting beta2-agonists (β2-agonists), long and short acting anticholinergics, inhaled corticosteroids and phosphodiesterase inhibitors are now the cornerstones of treatment for COPD.

Current guidelines

Once COPD is diagnosed, pharmacologic and non-pharmacologic interventions are recommended depending on symptoms and disease severity. The goals of pharmacologic therapy are to improve lung function, and quality of life while reducing daily symptoms, and exacerbation rates. The current Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines recommends the use of long-acting bronchodilators as first-line maintenance treatment for moderate COPD and beyond (6).

The prolonged duration of action of long-acting bronchodilators promotes medication adherence and compliance thus leading to improved clinical outcomes in COPD patients (5). Studies have shown that there is a strong correlation with medication adherence and dosing frequency (7). Currently available long-acting bronchodilators in the US include: twice-daily long-acting β-agonists (LABA), formoterol, arformoterol, and salmeterol; once-daily long acting anticholinergic (LAMA), tiotropium; twice daily LAMA, aclidinium, and indacaterol (5). Indacaterol is a once-daily LABA that was developed for maintenance therapy in patients with moderate to severe COPD. The European Union has approved indacaterol at a recommended dose of 150 µg once daily with a maximal dose of 300 µg once daily. In the United States, indacaterol is licensed to be used at a dose of 75 µg once daily (5).

Pharmacology

In the early twentieth century, epinephrine was introduced as a treatment of acute asthma. Modification of early catecholamine structures allowed for improved selectivity for the β2-adrenoreceptor, leading to the development of albuterol, a short acting β2-agonist (SABA). Eventually, there was development of longer-acting agents such as salmeterol and formoterol and finally the once-daily LABA, indacaterol. β2-agonists exert their effects via their ability to relax airway smooth muscle. They work by binding to active sites of β2-adrenoreceptors which are densely located on smooth muscle, resulting in the activation of adenylyl cyclase and the generation of intracellular cAMP (8). The increase in intracellular cAMP activates effector molecules such as cAMP-dependent protein kinase A which are involved in the regulation of airway smooth muscle tone (8).

Beta-agonists share a number of effects with epinephrine including: inhibiting airway smooth muscles; increasing heart rate, contraction and conduction; inhibiting mast cell degranulation; increasing glucose from glycogenolysis; increasing insulin and glucagon release, as well as, increasing or decreasing acetylcholine release (8).

β2-agonists are usually administered via inhalation, allowing for a direct route of drug delivery to the site of action at the lowest possible dose with the least systemic side effects. Studies show that the local drug concentration is the primary cause for β2-agonist's therapeutic effects, since peak plasma concentration only accounts for a small fraction of the decrease in airway resistance (8).

Pharmacokinetic reports show that indacaterol is rapidly absorbed via the pulmonary as well as the intestinal system, since a portion of the drug is always swallowed through the oropharynx into the gastrointestinal tract (8). *In vitro* studies show indacaterol has a median time to reach peak serum concentration of approximately 15 minutes after single or multiple doses. Another single-dose study of indacaterol at 600 and 2,000 µg confirmed a rapid absorption with maximal serum concentration reached within 15 minutes (5).

In vitro studies show that indacaterol has a high agonist efficacy at the human β2-adrenoreceptor with

a binding affinity similar to formoterol. Indacaterol has a functional selectivity to β2-adrenoreceptor over β1-adrenoreceptor that is similar to formoterol and over the β3-adrenoreceptors, which is similar to formoterol and albuterol (8). It is still unclear how LABAs are able to sustain a long bronchodilator effect. The previous thought of slow receptor dissociation does not appear to be the key to LABA's duration of action. It appears that the faster onset of action and longer duration of action of indacaterol is likely due to its lipid membrane interactions and ability for drug partitioning into lipophilic compartments upon inhalation (5,8). Another factor that may contribute to indacaterol's long duration of action is its high affinity for small lipid raft microdomains, which is the location where β2-adrenoreceptors are in close contact with effector molecules. It appears that indacaterol has a twofold higher affinity for these lipid rafts than salmeterol, which may contribute to its longer duration of action (8).

Toxicology evaluation of *in vitro* indacaterol shows no evidence of potential carcinogenicity and teratogenicity in embryo-fetal development. Multiple-dose studies of indacaterol at 400 and 800 μg for 14 days showed a rapid absorption and a mean elimination half-life >30 hrs (5).

Physiologic effects of indacaterol

Most COPD patients, especially those who continue to smoke, are known to lose lung function at an accelerated rate when compared with normal patients. Due to its ease of use and reproducibility, FEV1 is frequently used to assess this decline in lung function in order to monitor and direct appropriate treatment in COPD patients. Mean trough FEV1 has been shown to be proportional to change in health status of COPD patients (9). In one randomized placebo-controlled study over a 52-week period, all 366 patients on once-daily indacaterol at doses of 150 or 300 μg had a significant increase in FEV1 of greater than or equal to 170 mL (10). This was found to be statistically significant when compared with placebo. Another double-blind placebo controlled study of 90 patients who were treated with 300 μg of indacaterol once-daily for three weeks showed an increased FEV1 by 250 mL, which was statistically significant when compared with placebo (11). FEV1 measurements are helpful in determining treatment efficacy, however it does not reflect the full burden of COPD subjects (12).

Although FEV1 is an essential measure used in diagnosing, staging, and treatment of COPD, there have been studies showing that changes in FEV1 only partially correlate with changes in dyspnea. The mechanism of dyspnea in COPD is complex and multifactorial requiring monitoring of several factors including FEV1, functional vital capacity (FVC), and both static and dynamic lung hyperinflation to fully assess the benefit to COPD patients (13,14). Physiologic parameters such as FEV1, inspiratory capacity (IC) and total lung capacity (TLC) are used to diagnose and monitor treatment response in COPD patients (9). Static lung volumes such as IC have been purported to correlate better with exercise tolerance and dyspnea than FEV1 and FVC, as a measure of lung hyperinflation (11). A reduced ratio of IC and TLC has been shown to correlate with mortality predictions (15). This makes measurement of IC as a marker for severity and treatment response to COPD useful in treatment studies. In a study by O'Donnell, patients receiving 300 μg of indacaterol had an increase in IC of 170 mL at rest and an increase in end-exercise IC of 280 mL over placebo after three weeks of treatment (11). In another small randomized study in which 300 μg of indacaterol was used for three months as an add-on therapy, or replacement for other LABA, IC increased by 240 mL in the indacaterol treatment group. This study also showed a significant increase in six minute walk distance (6MWD), as well as, health related quality of life (HRQL) based on St. George's Respiratory Questionnaire (SGRQ) (16).

SGRQ is a well validated, disease-specific assessment developed to evaluate the health status of patients with asthma or COPD, and is frequently used in clinical trials as an endpoint to assess treatment effect in COPD (17). Health status and dyspnea, judged by measures such as TDI and SGRQ, were noted to be improved in a 52-week study by Dahl using 300 or 600 μg of indacaterol (16). Finally a meta-analysis on indacaterol with TDI data showed treatment doses of 150, 300 and 600 μg had significantly improvement in breathlessness in COPD patients when compared to placebo (18).

Safety

A safety study performed from data on multiple trials of indacaterol with once-daily dosing of 75, 150, 300, and 600 μg, elucidated five common adverse events. These include COPD worsening, nasopharyngitis, headache, cough, and upper respiratory tract infection (19). In this study, COPD worsening was found to be the most common adverse event, however its incidence was found to be less common on all

doses of indacaterol when compared with placebo. The other most common adverse events showed no consistent increase or decrease in incidence with indacaterol at all doses when compared with placebo (19).

Many different studies on indacaterol have mentioned coughing immediately following administration. This adverse effect was highlighted in a study by Donohue *et al.* where approximately 15-20% of patients had a cough starting within 15 seconds of drug administration and lasting less than 15 seconds, with a median duration of less than or equal to 6 seconds (19). Despite the frequency of immediate cough with medication, it was not associated with loss of efficacy, increased bronchospasm or discontinuation rates (20).

Cardiac side effects including tachycardia, QT prolongation, and palpitation have been frequently cited as a class-effect with beta-agonists. Other adverse reactions that are recognized consequences to beta2-adrenoreceptor stimulation include hypokalemia, and hyperglycemia, which can be detrimental to the heart. A RCT of 388 subjects on dosing of indacaterol up to 600 μg per day showed no clinically relevant effect on the QT interval (21). In a 52-week RCT by Chapman *et al.* with once-daily dosing of indacaterol, there was no effect on serum potassium level and only a slight increase in elevated blood glucose levels in the treatment group (10). Additionally an analysis of fvie RCTs showed that symptoms of anxiety, palpitations, and tachycardia were not increased with 150 and 300 μg dosing of indacaterol when compared with placebo (19). These findings show that indacaterol is well-tolerated at multiple doses with a good overall safety profile.

Jiang *et al.* showed that there was a dose response to adverse effects with indacaterol use. This was demonstrated with the development of more adverse events compared to placebo in a 52-week extended study of indacaterol 600 μg once-daily with risk ratio of 1.15. However it is also noteworthy that when compared with standard treatment of formoterol, a 52-week study found no more adverse events between the 600 μg of indacaterol and twice daily formoterol. Therefore, it seems that despite the adverse effects, a higher dose of indacaterol may provide better tolerance, improved patient compliance over the long term compared to formoterol (18).

Comparison studies

Several studies have been published to evaluate the bronchodilator effects and safety of indacaterol compared to currently available drugs.

Indacaterol vs. tiotropium

In 2010, Donohue *et al.* designed a randomized-controlled placebo study of 1,683 patients over a 26-week period to compare the efficacy of tiotropium and indacaterol. This was a two stage design, with initial two week stage used to determine the most efficacious dose of indacaterol by an independent committee. The 150 and 300 μg indacaterol doses were selected and compared with placebo and 18 μg of tiotropium. Spirometry was performed at baseline and at each visit. Dyspnea was evaluated based on the transition dyspnea index and health status was assessed with the SGRQ. At 12 weeks, there was an FEV1 difference versus placebo of 180 mL for both dose of indacaterol and 140 mL for tiotropium. At 26 weeks the difference in FEV1 over placebo was increased to 210 mL for 150 μg of indacaterol, 240 mL for 300 μg of indacaterol and 240 mL for tiotropium (*Figure 1*). Overall, indacaterol was well tolerated with headache being the most commonly reported adverse event which were generally mild to moderate. Additional adverse events include: tachycardia, which was noted in the lower dose of indacaterol; tremors, which were rarely reported, and prolonged QTc interval which were few in number and did not lead to any adverse events. Previous studies have shown a low arrhythmogenic potential for indacaterol. The study showed an improvement in dyspnea but not in health status based on mean SGRQ scores. Indacaterol maintained its bronchodilator effects over a 24-hour period of time. In this study it was felt that indacaterol was statistically noninferior to tiotropium (20). Other studies have confirmed that indacaterol is at least as effective as tiotropium and was able to show a statistically significant improvement in dyspnea and health status (based on SGRQ) with indacaterol compared to tiotropium (22).

Indacaterol vs. salmeterol

In 2011 Kornmann *et al.*, published a study comparing the efficacy and safety of indacaterol with salmeterol. This was a placebo-controlled study where patients were randomized to 150 μg of indacaterol once-daily, 50 μg of salmeterol twice daily or placebo over a 26-week period. A total of 838 patients completed the study with a significant difference compared to placebo noted in both indacaterol and salmeterol groups. The study showed a significant change in FEV1 over placebo of 170 mL at 12 weeks and 180 mL at 26 weeks in the indacaterol group and 110 mL at 12 and 26 weeks in the salmeterol group (*Figure 2*). There
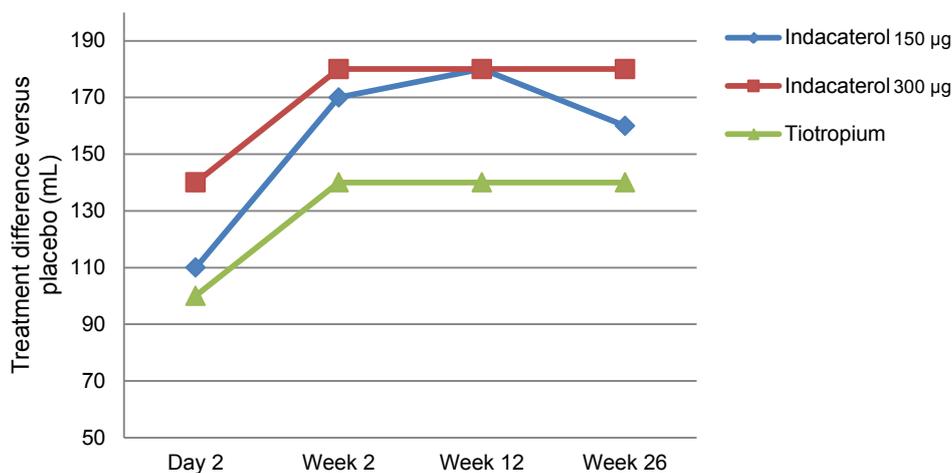
Figure 1 Differences between active treatments compared to placebo (mL) on trough forced expiratory volume in 1s (FEV1). Data are presented as least squares means. All treatment differences versus placebo were significant for superiority at P<0.001. Both indacaterol doses were significant for noninferiority versus tiotropium at P<0.001 (20).
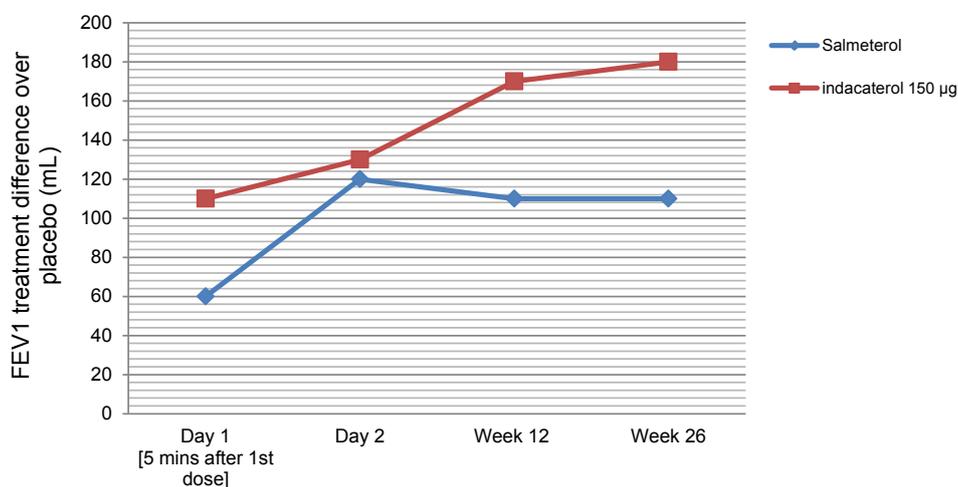
Figure 2 Differences between active treatment (salmeterol and indacaterol) over placebo for trough forced expiratory volume in 1s (FEV1). Data are presented as least squares means. Patient numbers analysed at day 2, week 12 and week 26, respectively, were 317, 320, and 300 (indacaterol), 320, 317 and 291 (salmeterol) and 321, 316, and 274 (placebo). Pre-specified 120 mL clinically important difference versus placebo. P<0.001 versus placebo. P<0.001 for indacaterol versus salmeterol (23).

appeared to a significant improvement in health status (based on SGRQ) at 12 weeks and improvement in dyspnea (based on TDI scores) at 4, 8, 12 and 26 weeks for indacaterol and 12 and 26 weeks for salmeterol. An evaluation done at 5 minutes after the first dose on the first day, showed an increase in FEV1 over placebo by 110 mL with indacaterol and by 60 mL with salmeterol. A similar improvement of 60-100 mL in FEV1 was observed throughout the study for indacaterol over salmeterol at 5 minutes. Adverse events noted in the study were similar across groups, with increased incidence of bacterial and viral upper respiratory tract infections in the indacaterol group (*Table 1*). Cough was reported on average 17.6% following inhalation of indacaterol which appears to occur within 15 seconds of

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Table 1 Adverse effects

	Indacaterol (%)	Salmeterol (%)	Placebo (%)
Subjects (n)	330	333	335
Pts with any adverse event(s)	169 (51.2)	152 (45.6)	156 (46.6)
COPD worsening	60 (18.2)	51 (15.3)	65 (19.4)
Nasopharyngitis	24 (7.3)	29 (8.7)	21 (6.3)
Upper respiratory tract infection			
Bacterial	14 (4.2)	3 (0.9)	5 (1.5)
Viral	10 (3.0)	3 (0.9)	7 (2.1)
Lower respiratory tract infection	9 (2.7)	13 (3.9)	8 (2.4)
Back Pain	7 (2.1)	12 (3.6)	6 (1.8)

Data are presented as n (%) unless otherwise stated. Most common events listed for >3% of the patients in either indacaterol or salmeterol groups. COPD, chronic obstructive pulmonary disease (23).

inhalation and lasted approx 12 seconds. This adverse effect did not appear to be associated with increase bronchospasm or loss of efficacy (23). This study shows that once-daily indacaterol is generally superior to twice-daily salmeterol and formoterol based on trough FEV1, improvement in health status, as well as, dyspnea (23,24).

Several studies have shown that indacaterol 150 and 300 μg dosing appeared to have similar lasting effects on trough FEV1. The higher dose of indacaterol, 300 μg, appears to have an incremental benefit in improvement in dyspnea (based on transition dyspnea index) over tiotropium, both twice-daily bronchodilators, as well as, patient with more severe COPD. The safety profile and tolerability of the different dosages appear to be similar (5). Patient using indacaterol had decrease use for as-needed SABA even when compared with tiotropium or formoterol. Although not statistically significant there was a reduction in the number exacerbation compared to placebo with use of all bronchodilators (5).

In 2011, Mahler *et al.* compared the efficacy of tiotropium monotherapy versus indacaterol 150 μg plus tiotropium in two identical double-blind randomized controlled studies over a 12 week period. Approx 1,100 patients with moderate to severe COPD were randomized in both studies. There was a statistically significant improvement in trough FEV1 of 80 and 70 mL with the combined treatment compared to monotherapy in each study respectively. This improvement was maintained in all subgroup analyses according to COPD severity, smoking status and inhaled corticosteroid use. Although the trough FEV1 was not as large as previous studies that compared indacaterol to tiotropium, there was an incremental improvement in FEV1 and IC which

shows that there is additional bronchodilator response with reduction in lung hyperinflation from combining these medications (25). The proposed mechanism of additive effects of bronchodilators includes: relaxation of smooth muscle secondary to independent effects on sympathetic and parasympathetic pathways, differential distribution of β2-adrenergic and muscarinic receptors, or the interaction of the two receptors causing a potentiation of β2-receptor activation by muscarinic receptor blockage (25).

A review article published in 2012 by Rodrigo *et al.* compared indacaterol (150-300 μg) with tiotropium or twice-daily LABAs. A total of five randomized controlled trials were reviewed which included approximately 6,000 participants. Two studies compared indacaterol with tiotropium and three studies compared indacaterol with twice-daily LABAs. Analysis revealed that there was no statistically significant improvement in trough FEV1 between indacaterol and tiotropium but there was a significant improvement of 80 mL in trough FEV1 with indacaterol and twice-daily LABAs. Compared with tiotropium and twice-daily LABA, indacaterol had a significant reduction in rescue albuterol use and sensation of dyspnea, which provides insight into the effectiveness of treatment. Compared with twice-daily LABA, indacaterol also had a significant improvement in health status, based on SGRQ. This study was able to quantify a number needed to treat with indacaterol of 10 patients in order to experience these clinical improvements over tiotropium or twice-daily LABAs. Although indacaterol showed improvement in health status, dyspnea and pulmonary function compared to tiotropium or twice-daily LABA, there was no significant difference in the rate of COPD exacerbation, withdrawal,

	Day 2		Week 12	
	Study 1	Study 2	Study 1	Study 2
■Placebo	1.27	1.37	1.25	1.35
■Indacaterol	1.36	1.45	1.38	1.49
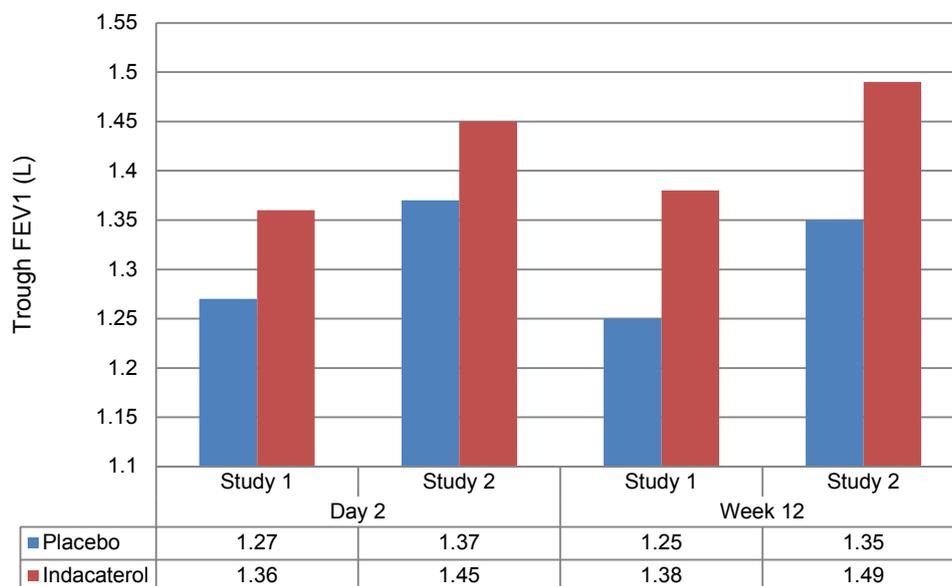
Figure 3 Trough forced expiratory volume in the first second of respiration (FEV1) after the first dose (assessed pre-dose on the morning of day 2) and after 12 weeks of treatment (primary end point). Data represent least squares mean (SE). P<0.001 versus placebo (29).

adverse events or all-cause mortality (26).

Indacaterol 75 µg dose

There has been some controversy regarding the most efficacious dose for the treatment of COPD. An analysis of 801 patients with moderate to severe COPD who were treated for 2 weeks showed that 150 µg of indacaterol was the lowest dose that was superior to the active comparators, formoterol and tiotropium (27). Despite these studies, the Food and Drug Administration (FDA) approved indacaterol at a dose of 75 µg once daily. According to the FDA's analysis of the original dose-exploration data, all doses on indacaterol fulfilled the criteria of trough FEV1 greater than 0.12 liters over placebo, as well as, produced a higher trough FEV1 with an area under the FEV1 curve 1 to 4 hours after a dose compared with other bronchodilators (28). It was felt that all doses were more effective than placebo without a significant increase in dose-response above the 75 µg dose. A 12 week placebo controlled study was performed by Novartis to compare the 75 and 150 µg doses of indacaterol; both dosages resulted in a significantly higher trough FEV1 than placebo. The study also showed a pooled analysis for HRQL (based on SGRQ) at week 12 showing a statistically significant improvement with 75 µg dose of indacaterol over placebo, but higher doses did not show any incremental

benefits in quality of life. An analysis of 23 trials with over 11,755 COPD patients did not show any significant worrisome findings for indacaterol at the 75 and 150 µg doses, but there was some concern for increase asthma exacerbation and respiratory related deaths with the 300 µg of indacaterol. Due to this risk-benefit evaluation, the FDA approved the use of 75 µg dose of indacaterol in the United States (27).

Kerwin *et al.*, published two identical studies in 2011 comparing the efficacy of indacaterol 75 µg with placebo in a double-blind placebo-controlled study over a 12-week period. Each study evaluated approximately 320 patients and they both showed a significant improvement in trough FEV1 in the indacaterol group over placebo. The increase in trough FEV1 over placebo at 12 weeks was 120 and 140 mL for each study respectively (*Figure 3*). The 75 µg indacaterol dose also confirmed a rapid onset with prolong duration of action; the 5 minutes post-dose on day one was approximately 90-100 mL which had sustained efficacy throughout the day (*Figure 4*). Approximately half of the patients in both studies reported adverse events. The most common event in each group, which were similar to previous studies, included COPD worsening, an acute cough, headache, nasopharyngitis and rarely tachycardia or muscle spasm. Although there were two deaths in the placebo group, this was not felt to be related to the study

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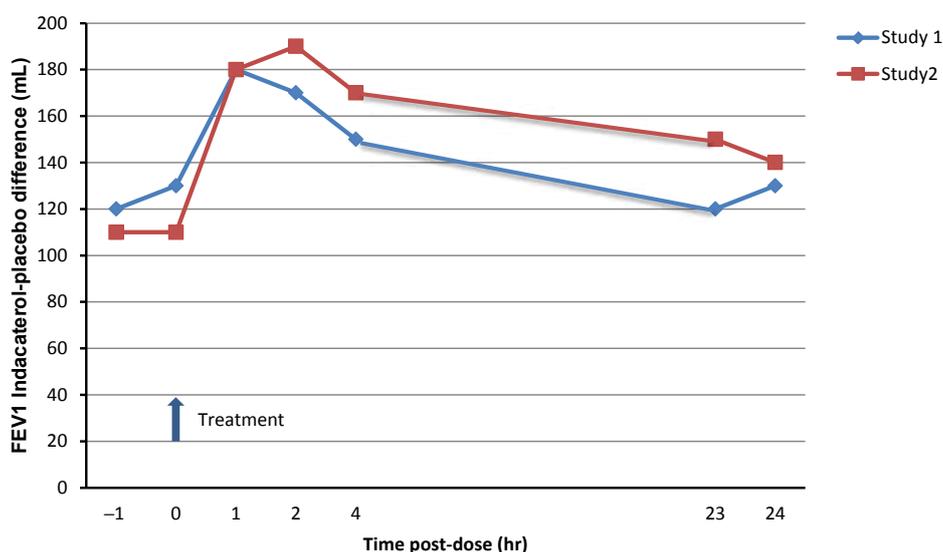
Figure 4 Indacaterol-placebo difference in forced expiratory volume in the first second of respiration (FEV1) at post-dose time points (1, 2, 4 hrs, 23 hrs 10 min, and 23 hrs 45 min) at week 12. Data represents least square means. All indacaterol-placebo differences were significant at P<0.001 (29).

medication. This dose of indacaterol was also able to show a significant reduction in use of rescue albuterol showing its effectiveness to control symptoms (29).

A follow-up study by Gotfried *et al.*, was published in 2012 showing the secondary efficacy endpoints from the two double-blind, placebo-controlled 12 weeks studies published by the same authors. Both studies showed a statistically significant improvement in dyspnea, measured by TDI, at 4 weeks but only in one study at 12 weeks. Health status, measured by the SGRQ, showed a clinically relevant improvement with indacaterol from baseline across all domains. Both studies showed numerical improvement in various symptom-based end-points, a few of which were statistically significant including percentage of days able to perform usual activities, percentage of days with no daytime symptoms, percentage of nights with no awakenings, as well as, overall number of COPD exacerbations. The use of rescue albuterol use was statistically significant with indacaterol in both studies. Although not specifically powered for these end-points, these studies show a clinically relevant improvement in health status, based on SGRQ scores, compared to baseline (30).

In 2012, Cope *et al.* published a meta-analysis comparing indacaterol 75 µg with fixed-dose combinations of formoterol-budesonide or salmeterol-fluticasone. Based on the studies, indacaterol 75 µg resulted in a greater improvement in trough FEV1 at 12 weeks compared to formoterol-budesonide at doses of 9/160 and 9/320 µg twice-daily. This meta-analysis also showed that indacaterol 75 µg had a comparable change in trough FEV1 from baseline compared with salmeterol-fluticasone at 50/250 and 50/500 µg twice-daily doses. They concluded that indacaterol at 75 µg was at least as efficacious as formoterol-budesonide at both dosages and is comparable with salmeterol-fluticasone at both dosages (31).

In 2012, Cope *et al.* published another meta-analysis comparing 75 µg indacaterol with usual doses of tiotropium, salmeterol, formoterol, and placebo. Overall, there were 22 randomized controlled trials that were included in the analysis. The results of the meta-analysis showed that 75 µg of indacaterol provides an FEV1 result that was comparable to tiotropium and salmeterol at 12 weeks and a higher FEV1 versus formoterol. With regards to assessment of HRQL, based on SGRQ, all treatments were more efficacious than placebo at 12 weeks. This shows that there is a comparable level of improvement in HRQL with indacaterol 75 µg compared to tiotropium, salmeterol and formoterol (32).

Cost-effectiveness

Due to the large number of variables related to medication costs, including differing health policies,

health insurance costs, medication compliance rates, pharmaceutical/commercial aspects and patient preferences, determining the cost-effectiveness of a medication is extremely difficult. Current data appears to indicate there is a difference in cost-effectiveness between bronchodilators. A recent cost-effectiveness analysis from Germany of indacaterol showed that indacaterol 150 μg is better (lower total cost with better outcomes) than tiotropium bromide or salmeterol. Another analysis comparing indacaterol 300 μg against tiotropium showed an incremental cost-effectiveness ratio of approximately 28,300 euros per quality-adjusted life years (33).

Conclusions

Indacaterol is a once-daily long-acting β2-agonist, approved for the treatment of moderate to severe COPD. It is currently approved in the United States at a dose of 75 μg and in the European Union at a recommended dose of 150 μg as well as 300 μg for severe COPD. Studies show that it has a rapid onset of action, within 5 minutes, and provides prolonged bronchodilator effects, at least 24 hours. Current studies show that indacaterol is as effective as tiotropium and superior to twice-daily LABA, salmeterol and formoterol. Higher dosing of indacaterol did provide some incremental benefit associated with improvement in trough FEV1, and dyspnea, however some studies showed a trend towards increased adverse effects. The improvement in lung measurements correlate to clinical outcomes, including improvement in dyspnea, HRQL, and exacerbation rate when compared to some of the other available bronchodilators, but mainly when compared to placebo. Several studies show that indacaterol is generally well tolerated and has a favorable safety profile when compared to other bronchodilators without significant safety issues. The most prevalent side effect is a cough which is seen within seconds of inhalation with rapid resolution. This adverse effect does not appear to cause bronchospasm or loss of efficacy. Other relevant adverse effects include: worsening COPD, upper respiratory infection, and possible increase in asthma exacerbation, and asthma-related deaths, particularly at higher doses of indacaterol. There were no significant or clinical relevant cardiac effects, including QTc prolongation, tachycardia, high blood pressure, hypokalemia or hyperglycemia. Since current guidelines recommend at least one long-acting bronchodilator as first-line maintenance therapy for moderate symptomatic COPD

patients, it seems that both indacaterol, a once-daily LABA, or tiotropium, a once-daily LAMA, would be an appropriate starting medication. The once-daily dosing will provide long acting bronchodilation and promote medication compliance in order to improve patient outcomes. If symptoms are not controlled with a once-daily bronchodilator, GOLD guidelines recommend combining a LABA and LAMA. The use of indacaterol as add-on therapy has been shown to provide additional clinical improvement which will allow for a convenient treatment regimen compared to what most COPD patients currently use. This review shows that indacaterol is appropriate first-line or additive treatment option for the management of moderate to severe COPD (GOLD stage II-III) and, although not specifically studied, will likely be beneficial for late stage COPD (GOLD stage IV).

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Targeting pro-resolution pathways to combat chronic inflammation in COPD

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Abstract: Chronic obstructive pulmonary disease (COPD) is an inflammatory lung condition that is associated with irreversible airflow obstruction as a consequence of small airways disease, excessive mucus production and emphysema. Paradoxically, excessive inflammation fails to control microbial pathogens that not only colonise COPD airways, but also trigger acute exacerbations, which markedly increase inflammation underlying host tissue damage. Excessive production of leukocyte mobilising cytokines such as CXCL8 (IL-8) and leukotriene B4 (LTB_4) in response to environmental stimuli (cigarette smoke and microbial products) are thought to maintain chronic inflammation, in conjunction with inefficient macrophage clearance of microbes and apoptotic neutrophils. In this perspective, we discuss an alternative view on why inflammation persists with a focus on why pro-resolution mediators such as lipoxin A4 (LXA_4), D-series resolving and Annexin A1 fail to effectively switch off inflammation in COPD. These pro-resolving mediators converge on the G-protein coupled receptor, ALX/FPR2. This receptor is particularly relevant to COPD as the complex milieu of exogenous and host-derived mediators within the inflamed airways include agonists that potently activate ALX/FPR2, including Serum Amyloid A (SAA) and the cathelicidin, LL-37. There is emerging evidence to suggest that ALX/FPR2 can exist in alternative receptor conformations in an agonist-biased manner, which facilitates alternate functional receptor behaviors. Hence, the development of more stable pro-resolving analogs provides therapeutic opportunities to address ALX/FPR2 conformations to counteract pathogenic signaling and promote non-phlogistic clearance pathways essential for resolution of inflammation.

Keywords: ALX/FPR2; chronic obstructive pulmonary disease (COPD); inflammation; catabasis; resolution; Serum Amyloid A (SAA)

Introduction

Chronic obstructive pulmonary disease (COPD) is a major global health issue and is a leading cause of morbidity and mortality worldwide (1). Cigarette smoke exposure is a primary cause of COPD and as smoking rates continue to rise in South-East Asia, the long disease latency of COPD will impact dramatically on global health care resources for many decades to come. It is also well established that environmental influences such as indoor and outdoor air pollutants contribute to the development of chronic lung disease in individuals with no smoking history (2). Pathologically, COPD manifests into impaired lung function and irreversible airflow obstruction as a consequence of small airways disease, excessive mucus production and the development of emphysema. The mechanisms that drive such deleterious remodelling processes are heterogeneous in nature; however it is evident that chronic inflammation and excessive oxidative stress become central to the progression of COPD (3).

Nature of persistent inflammation in COPD

Innate and adaptive immune cells accumulate with

progression of COPD. CD8+ T cells increase with disease severity and can release proteolytic enzymes that contribute to apoptosis of structural cells (4). CD4+ T cells, B cells and dendritic cells also aggregate into organised tertiary lymphoid organs (TLOs), which are found in COPD lungs and experimental models of chronic cigarette smoke exposure (5). The significance of these lymphoid follicles has yet to be established, where B cell accumulation may prove to be beneficial in terms of antibody production to colonising and invading pathogens, or detrimental through autoantibody production against lung tissue antigens (6). Of note, targeted disruption of TLO formation through neutralisation of CXCL13 does not reduce lung inflammation and alveolar enlargement in a chronic smoke exposure model (7), suggesting that immune cells of the innate system are sufficient to drive pathological changes that occur in COPD. In addition, inflammation does not fully resolve even when individuals with COPD stop smoking. Inflammation persists in bronchial biopsies and in sputum samples from COPD patients when compared to asymptomatic smokers (8-10). In particular, neutrophilic inflammation remained elevated in COPD subjects who had ceased smoking for at least one year (8-10). Given that neutrophils are a relatively short-lived immune cell; their persistence in COPD indicates that there is continual recruitment of leukocytes into the airways even when the primary insult of smoke exposure is removed.

Smoke exposure is particularly deleterious to host immunity against respiratory pathogens. Macrophage function is compromised in a microenvironment where there is excessive oxidative stress, leading to a deficiency in phagocytosis of bacteria (11) and efferocytosis of apoptotic cells (12). Free radicals have been shown to impair clearance mechanisms by directly causing cytoskeletal instability and carbonyl modification of pseudopodia (13-15). Macrophages also interact with carbonyl-adduct modified extracellular matrix proteins, which impair their ability to clear apoptotic neutrophils (16). Reduced macrophage function is consistent with high colonisation rates in COPD, where up to 50% of COPD patients are chronically colonized with microorganisms including Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catarrhalis (17,18). Impaired immunity to respiratory pathogens also contributes to susceptibility to acute exacerbations of COPD (AECOPD), which are predominately caused by a new respiratory pathogen that is different to the colonized pathogen (19). AECOPDs result in a more rapid decline in lung function (20), which adversely affects mortality rates (21)

and health related quality of life (22). Viral infections are a common cause of AECOPDs (23,24), as are bacterial infections, which are associated with a marked increase in neutrophilic inflammation (25).

Bacterial colonization and AECOPDs contribute to persistent inflammation of the airways, as neutrophils and monocytes are recruited from the circulation in an attempt to clear the invading pathogens. Since resident airway macrophages in COPD have a reduced capacity to clear efferocytic neutrophils, the emergence of necrotic neutrophils may further facilitate a vicious cycle of inflammation through release of endogenous damage associated molecular patterns (DAMPs). For example, degranulating necrotic bodies release neutrophil elastase, which localize to lung elastic fibers in emphysematic patients and degrades extracellular matrix components (26). Excessive neutrophil elastase activity can also promote the release of mucins through Epidermal Growth Factor Receptor (EGFR)-dependent mechanisms (27). Furthermore, increased EGFR transactivation augments inflammatory responses initiated by rhinovirus infection in bronchial epithelial cells (28). Necrotic neutrophils also release High-Mobility Group Box-1 (HMGB1), which is a DNA binding protein that is elevated in COPD airways (29). HMGB1 can synergize with microbial products and endogenous cytokines to enhance inflammation through Toll Like Receptor (TLR) (30,31) and Receptor for Advanced Glycosylation End (RAGE) (29) dependent mechanisms. Hence, COPD airways represent a highly complex milieu consisting of inhaled irritants, respiratory pathogens and endogenous mediators released from damaged tissue, which collectively drive excessive host immunity and sterile inflammation.

Pro-resolution mediators promote catabasis in the inflamed airway

The ongoing recruitment of leukocytes from the circulation is consistent with increased local production of CXCL chemokines in COPD including IL-8 (CXCL8), ENA78 (CXCL5), GCP-2 (CXCL6) and GRO isoforms (CXCL1-3) (32,33). The CXCR family of G coupled protein receptors (GPCR) binds to these endogenous chemokines, where CXCR2 is the cognate receptor for this family (34). There is also a high degree of redundancy in leukocyte mobilization as alternate mediators such as leukotriene B4 (LTB4) are associated with neutrophilic inflammation in colonized COPD patients (35). These

pathways are essential to lung host immunity and are normally self-limiting through the activation of pro-resolution pathways. Pro-resolving mediators actively counterbalance inflammation by effectively switching off mechanisms that maintain leukocyte recruitment and survival. Eicosanoids such as lipoxins, resolvins and protectins and the protein termed Annexin A1 are integral to resolution of inflammation, [reviewed in (36-38)]; where there is a class switch of eicosanoid production from chemoattractants (such as the leukotriene LTB4) to pro-resolving mediators (such as lipoxin A4, LXA4) (39).

Lipoxins are synthesized in response to cell-cell interactions [reviewed in (40,41)]. In the lung, 15-lipoxygenase is expressed in epithelial cells and converts arachidonic acid to 15S-hydroxyleicosatetraenoic acid (15S-HETE). This metabolite is then taken up by neutrophils and converted to LXA4 by the enzyme 5-lipoxygenase (42). LXA4 opposes leukocyte migration and activation through multiple mechanisms including suppression of transendothelial (43) and transepithelial (44) migration and azurophilic degranulation (45). LXA4 can also directly target activated mucosal epithelial cells by opposing production of inflammatory cytokines (46). *In vivo*, administration of a stable analog of LXA4 reduced pulmonary inflammation and airway hyperreactivity in a murine model of asthma (47). Furthermore, this eicosanoid can directly contribute to tissue repair through the promotion of basal cell proliferation required for wound healing following mucosal injury (46). Another important anti-inflammatory role for LXA4 is its influence on macrophage function. Treatment with nanomolar concentrations of LXA4 stimulated more efficient efferocytosis of apoptotic neutrophils in human monocyte derived macrophages without provoking release of inflammatory mediators (48,49). LXA4-induced uptake of apoptotic neutrophils was reduced in macrophages from Fpr2 deficient mice, which is the mouse ortholog of the human ALX/FPR2 G-protein coupled receptor (GPCR) that potently interacts with lipoxins (50).

ALX/FPR2 is central to resolution of inflammation

In addition to lipoxins, the alternate anti-inflammatory mediators Annexin A1 and Resolvin D1 also interact with ALX/FPR2, implicating this receptor as an integral component of pro-resolution pathways. Accordingly, they display overlapping functions, where both LXA4 (51) and Annexin A1 (52) promote apoptosis of neutrophils as a mechanism of resolving acute inflammatory responses. Unlike lipid mediators derived from cell-cell interactions, Annexin A1 is highly abundant in neutrophils where the 37kDa protein is localized to the cytoplasm and in gelatinase/azurophilic granules. Annexin A1 is a glucocorticoid-induced lipocortin that can be rapidly mobilized through degranulation of neutrophils. Cytoplasmic Annexin A1 is also subjected to post translational modifications such as phosphorylation, which facilitate translocation to the cellular membrane [reviewed in (52)]. In addition to promoting neutrophil apoptosis, Annexin A1 reduces neutrophil endothelial attachment and subsequent transmigration. Like LXA4, Annexin A1 has also been shown to enhance efferocytosis of apoptotic neutrophils by macrophages [reviewed in (52)], thereby facilitating catabasis of the inflamed tissue to homeostasis.

Another family of mediators that target ALX/FPR2 is the D series resolvins derived from the omega-3 fatty acid, docosahexaenoic acid (DHA) [reviewed in (53)]. Although there is relatively less information on the actions of D-series resolvins in lung diseases, there is emerging evidence to support an important role in resolution of lung inflammation. In a murine model of acute lung injury, treatment with stable derivatives of Resolvin D1 improved epithelial and endothelial integrity and reduced neutrophilic inflammation concurrently with reduced inflammatory cytokine secretion in the airways (54). In addition, aspirin-triggered Resolvin D1 (AT-RvD1) significantly reduced airway inflammation and enhanced macrophage phagocytosis in a murine asthma model (55). Furthermore, Resolvin D1 reduced neutrophilic lung inflammation, inflammatory cytokine production and phagocytosis in an acute cigarette smoke exposure model (56).

Is there an imbalance between inflammatory and pro-resolving mediators in COPD?

Given their important role in resolution of acute inflammation, any perturbation in the production of pro-resolving mediators can lead to the generation of pathogenic inflammatory conditions. Indeed, it has been shown that reduced production of LXA4 relative to cysteinyl leukotrienes is associated with the persistence of inflammation in severe asthma (57,58). Reduced levels of LXA4 are also observed in exhaled breath condensate during asthma exacerbations (59) and in the airway fluid of patients with cystic fibrosis (60). Since multiple mediators converge on ALX/FPR2, their relative abundance within the airway milieu of the chronically inflamed lung can profoundly

influence catabasis and restoration of homeostasis.

ALX/FPR2 belongs to the N-formyl peptide receptor (FPR) family, of which there are three human members (FPR1, ALX/FPR2 and FPR3). FPRs demonstrate wide tissue distribution, although ALX/FPR2 expression is particularly prominent on myeloid cells including neutrophils and monocytes (61). Airway mucosal epithelial cells also express ALX/FPR2 in a manner that is increased via COX-2 dependent mechanisms in response to injury (46). Prominent staining is also observed on the apical and basolateral side of the epithelium of COPD airways (62).

ALX/FPR2 is a GPCR superfamily member characterized by seven putative TM domains that displays diverse ligand affinities that extend beyond interactions with lipoxins, series D-resolvins and Annexin A1. Accordingly, ALX/FPR2 can interact with over 30 ligands that can exert opposing biological actions (63). Hence, selective agonists are likely to bind with different affinities and given the diverse conformation of endogenous and synthetic ligands, are likely to bind to alternate regions of the receptor. Numerous receptor conformations can exist where ligand binding alters receptor formation, which facilitates alternate functional behaviors that control multiple downstream signaling pathways (64). This is true for ALX/FPR2, where agonist biased signaling can either promote inflammation or resolution contingent on alternate receptor conformations [reviewed in (65)]. Serum Amyloid A (SAA) and the anti-microbial peptide LL-37 both interact with ALX/FPR2 and in complete contrast to pro-resolving mediators, promote neutrophil transmigration, activation and survival (summarized in *Figure 1*). Another high affinity ligand for ALX/FPR2 includes mitochondrial N-formylated hexapeptides derived from NADH dehydrogenase and cytochrome c oxidase subunits (66). These formylated peptides are released from damaged cells and can interact with FPR1 and ALX/FPR2 (66). Although it is not known whether these ligands are elevated in COPD, there is increased mitochondrial dysfunction and cytochrome c oxidase levels in the skeletal muscle of COPD patients (67-69).

There are multiple mechanisms that may address why ALX/FPR2 receptors modulate cell responsiveness in a ligand-biased fashion. This includes the formation of homologous and/or heterologous receptor dimers and differential conformational ligand activation of specific receptor domains. The molecular nature of how ALX/FPR2 downstream signaling pathways are activated in a ligand-specific manner has yet to be fully characterized. However, there is evidence for conformational ligand activation.

LXA4 has been shown to activate ALX/FPR2 by interacting with extracellular loop III and the associated transmembrane region (70), whereas SAA initiates extracellular loops I and II dependent signaling (71). In addition, SAA has recently been shown to promote ALX/FPR2 homodimerisation and activation of pro-survival pathways, which can be counteracted by Annexin A1 that promotes an alternative receptor conformation that engages pro-apoptotic pathways (72).

Conclusions

Since the accurate detection of lipid pro-resolution mediators in human tissue requires LC-LC-MS approaches that have only recently become available, there are currently relatively few studies demonstrating their presence in human tissue (73). Hence, there is a current knowledge gap in terms of whether there is a deficiency in production of pro-resolving mediators in COPD that facilitates a chronic inflammatory state, and advances in detection technology will reveal new insight here. Importantly, this should inform on the development of new classes of drugs to treat COPD away from conventional steroid-like anti-inflammatory approaches that can detrimentally dampen immunity leading to immunosuppression. Specialized pro-resolving mediators offer an alternative approach to switching off chronic inflammation and may concurrently boost beneficial host immunity and tissue repair mechanisms. Whilst the organic synthesis of pro-resolving lipid mediators can be challenging, chemically stable E-class resolvin analogs have been successfully synthesized and are currently in Phase III clinical trial for chronic inflammatory dry eye syndrome (Resolvyx Pharmaceuticals in partnership with Celtic Therapeutics).

Of interest, two human phenotypes have been described in response to cantharidin-induced skin blisters in male healthy volunteers involving distinct production of LXA4, which controlled the longevity and severity of the inflammation (74). The relative abundance of alternative ALX/FPR2 ligands may also directly influence the functional behavior of this complex receptor in COPD airways, where there is a rich milieu of microbial derived products and endogenous mediators. LL-37 (or hCAP-18) is a breakdown product of cathelicidin that promotes microbial killing through pore formation. LL-37 is also a chemoattractant for neutrophils, monocytes and lymphocytes via its actions on ALX/FPR2 (75). Since LL-37 levels are elevated in sputum samples from stable COPD patients (76) and are further increased during
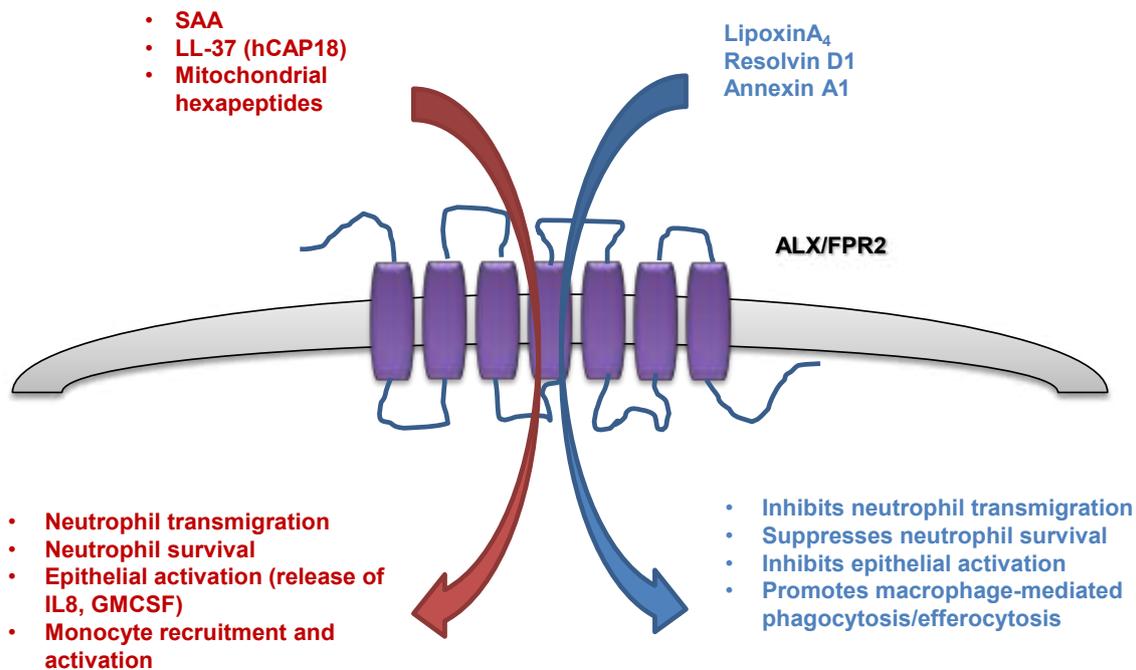
Figure 1 ALX/FPR2 and chronic inflammation in COPD. The ALX/FPR2 complex is expressed on leukocytes and the respiratory mucosa, where expression is particularly prominent on injured epithelial cells. The expression of two known ligands for ALX/FPR2, Serum Amyloid A (SAA) and LL-37 (hCAP-18) are known to be elevated in COPD and acute exacerbations of COPD. Mitochondrial hexapeptides released from damaged tissue also activate ALX/FPR2. SAA and LL-37 have been shown to initiate a pro-inflammatory response involving recruitment of neutrophils and monocytes. SAA can also promote neutrophil survival and release of inflammatory mediators from epithelial cells. In contrast, alternate ligands can bind to a different receptor region and promote distinct receptor conformations that translate to an opposing biological action. When the pro-resolving mediators LipoxinA4, Resolvin D1 and Annexin A1 interact with this receptor, they can actively initiate processes to reduce the influx of leukocytes and stimulate macrophages to clear exhausted immune cells and damaged tissue. SAA is disproportionally expressed relative to LXA4 in circulation during exacerbations and with the recent advance in technology to detect pro-resolving mediators in human tissue; it will be possible to explore this putative imbalance in COPD airways. With the ongoing development of more stable pro-resolving analogs, their therapeutic application may provide an opportunity to suppress chronic inflammation and clear damaged tissue to promote catabasis in the lung.

bacterial AECOPDs (77), this normally protective molecule may contribute to persistent inflammation. Likewise, SAA, which has previously been characterized as a systemic biomarker for AECOPD severity (78), is prominently expressed in lung resection tissue from COPD patients (62). Using the same lung resection tissue, SAA transcript expression was also detected and found to be positively associated with the number of tissue neutrophils (79).

In addition to promoting neutrophil survival via ALX/FPR2, SAA is a potent chemotactic factor that mediates migration of leukocytes (80) and can also stimulate expression of pro-inflammatory mediators under *in vitro* (81) and *in vivo* conditions (62). More recently, SAA was shown to promote the differentiation of monocyte derived

macrophages into a pro-inflammatory phenotype that expresses higher levels of the TH17 polarizing cytokines, IL-6 and IL-1β in a manner that was dependent on CSF-1R signaling (82). Hence, the relative abundance and persistence of pro-inflammatory agonists such as SAA and LL-37 in COPD airways is likely to facilitate agonist biased signaling that favors leukocyte recruitment, activation and survival. The nature of this receptor also provides therapeutic opportunities to address ALX/FPR2 conformations that may lead to pathogenic functions through the development of more stable analogs of lipoxins, D-series resolvins and cleavage resistant Annexin A1 mimetics, which can override pro-inflammatory signals to initiate resolution of inflammation.

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Central sleep apnoea—a clinical review

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Abstract: Central sleep apnoea (CSA) is characterised by recurrent apnoeas during sleep with no associated respiratory effort. It mostly results from withdrawal of the wakefulness drive in sleep leaving ventilation under metabolic control. A detailed physiological understanding of the control of breathing in wakefulness and sleep is essential to the understanding of CSA. It encompasses a diverse group of conditions with differing aetiologies and pathophysiology. Likewise treatment varies according to underlying aetiology. Some of the conditions such as idiopathic (primary) CSA (ICSA) are relatively rare and benign. On the other hand Cheyne-Stokes breathing (CSB) pattern is quite common in patients with heart failure and might be a prognostic indicator of poor outcome. Unfortunately modern medical management of heart failure does not seem to have significantly reduced the prevalence of CSA in this group. Since the adoption of positive airway pressure (PAP) as a common treatment modality of obstructive sleep apnoea (OSA), complex CSA has been increasingly observed either as treatment emergent or persistent CSA. Depending on the particular condition, various treatment strategies have been tried in the past two decades which have included hypnotic therapy, respiratory stimulants, judicious administration of carbon dioxide, oxygen therapy, PAP and bi-level ventilatory support with a backup rate. In the past decade adaptive servo ventilation (ASV) has been introduced with much promise. Various studies have shown its superiority over other treatment modalities. Ongoing long term studies will hopefully shed more light on its impact on cardiovascular morbidity and mortality. Other rare forms are still poorly understood and treatments remain suboptimal.

Keywords: Apnoea; breathing control; servo-ventilation

Introduction

Central sleep apnoea (CSA) is characterised by recurrent apnoeic episodes with no associated respiratory effort. Compared to obstructive sleep apnoea (OSA) it is not as commonly seen in sleep centres accounting for about 5-10% of clinic patients. It is, however, quite common in patients with heart failure, in patients with some neurological disorders and in those on high dose opiates. Complex CSA is now well recognised in continuous positive airway pressure (CPAP) treated OSA. Symptoms of CSA include sleep fragmentation, insomnia and daytime hypersomnolence, which is usually not as severe as in OSA. Despite a lot of research into CSA in the past two decades the condition is still poorly understood and treatment is still suboptimal perhaps owing to its aetiological and pathophysiological diversity and heterogeneity.

Definitions and classifications

CSA in adults is defined as cessation in airflow of 10 or more seconds in the absence of any inspiratory effort. CSA is diagnosed when 50% or more of the events are central. In routine practice oro-nasal thermal sensor signals are used to detect apnoeas. More accurate determination of absence of inspiratory effort would entail use of an oesophageal balloon catheter. Respiratory inductance plethysmography and strain gauges are alternative measures as is diaphragmatic electromyography. A frequency of 5 or more apnoeas per hour is considered abnormal.

The *International Classification of Sleep Disorders*, 2nd

Box 1 Central sleep apnoea (CSA) syndromes
Primary (idiopathic) CSA
Cheyne-Stokes breathing pattern
CSA due to high-altitude periodic breathing
CSA due to drug or substance
Primary sleep apnoea of infancy
From the American Academy of Sleep Medicine: ICSD-2 2005 (1). ICSD, International Classification of Sleep Disorders.

edition (ICSD-2) lists five CSA syndromes (*Box 1*) (1).

As suggested by Bradley *et al.* it is useful to divide the CSA syndromes into two broad groups: the hypocapnic and the hypercapnic groups (2). Patients in the hypocapnic group have normal or low wake $PaCO_2$ and do not develop sleep hypercapnoea. This group includes patients with ICSA, Cheyne-Stokes breathing (CSB) pattern and complex CSA. The hypercapnic group has wake high normal or elevated wake $PaCO_2$ which may rise further in sleep. This group includes patients with CSA due to drug or substance and primary CSA of infancy. This group also includes patients with obesity hypoventilation syndrome, thoracic cage disorders, neuromuscular disorders and other hypoventilation syndromes which will not be discussed further in this article.

Complex sleep apnoea (complex SA) refers to patients who have primarily OSA or mixed apnoeas who develop central apnoeas on positive airway pressure (PAP) treatment (treatment-emergent central apnoeas) or have significant persistent central apnoeas on PAP treatment (treatment-persistent CSAs).

Pathophysiology of CSA

Breathing in non-rapid eye movement (NREM) sleep

An understanding of the normal physiological changes in breathing between wake and NREM sleep is essential for the understanding of CSAs. Respiratory control is quite unstable during transition from wake to sleep and with further sleep stage transitions (3,4). There is usually a 2 to 8 mmHg rise in $PaCO_2$ during NREM sleep. During NREM sleep ventilation is totally under metabolic control (5). The wakefulness drive is no longer operational, the hypercapnic and hypoxic drives are reduced and there is also increased upper airway resistance (6-8). But is it really as simple as this? Some doubt has been raised as to whether there is true loss of chemo-responsiveness and loss of drive

and some have suggested that the changes in sleep might be mostly due to increased upper airway resistance.

Breathing in REM sleep

REM sleep is characterised by generalised skeletal muscle atonia leaving the diaphragm as the main active respiratory muscle. There is further reduction of the hypercapnic and hypoxic drives. There is also further inhibition of upper airway muscles and diaphragmatic activity in phasic REM which may lead to episodes of CSA and reduced tidal volumes (9-11).

Apnoeic threshold (AT)

If the $PaCO_2$ falls below an individual's set value (apnoea threshold) a CSA will ensue. Ventilation will only resume after the $PaCO_2$ has risen above that threshold. The sleeping $PaCO_2$ is normally 2 to 8 mmHg above the wake level. The AT is usually 1 to 2 mmHg lower than the waking $PaCO_2$. The important factor which determines the propensity to develop an apnoea is the difference between the individual's $PaCO_2$ and the AT. The smaller the $PaCO_2$-AT difference the more likely it is that a central apnoea will occur. It is important to note that an individual's $PaCO_2$-AT difference is not fixed and can vary with ventilatory drive (12).

Loop gain

The engineering concept of loop gain is helpful in the understanding of the pathogenesis of CSA. The loop gain is determined by the plant gain and controller gain. In this case the plant gain is the ability to increase ventilation by the lungs and respiratory muscles and the controller gain the change in ventilation induced by a change in $PaCO_2$.

Idiopathic (primary) CSA (ICSA)

The aetiology and pathogenesis of ICSA is still not clear. It is essentially a diagnosis of exclusion after excluding the known causes of CSA. Patients are usually not obese. Symptoms include fragmented/disturbed sleep, insomnia, witnessed apnoeas and some daytime sleepiness. Snoring is not as intrusive as in OSA. These patents tend to have a high hypercapnic response (2,13,14) and sleep state instability. Wake $PaCO_2$ levels tend to be low. Arousals, usually non-respiratory related, cause a transient increase in ventilation and a consequent fall in $PaCO_2$. Unlike Cheyne-

Stokes there is no waxing and waning of ventilation. Polysomnographic studies usually show frequent isolated central apnoeas or runs of central apnoeas. They occur in NREM sleep and mostly arise from stage 1 and 2. Treatments which have been tried include respiratory stimulants, hypnotics, CPAP and oxygen therapy (15). The carbonic anhydrase inhibitor acetazolamide which induces a metabolic acidosis has been shown to reduce AHI by as much as 50% but has a doubtful impact on sleep efficiency (16). Hypnotics work by suppressing the arousal response and consolidating sleep (17-19). Stage 3 NREM sleep is increased, $PaCO_2$ levels are higher and respiration is more stable. In some patients hypnotics can increase obstructive events; they should not be used in patients with hypercapnic CSA. CPAP has been used to treat ICSA (20,21). It probably works by stabilising the upper airways and abolishing the upper airway resistance related arousals. It might also minimise $PaCO_2$ overshoots after an arousal. It has also been suggested that it might slightly increase the sleeping $PaCO_2$ in patients who are hypocapnic at baseline. Xie et al. demonstrated elimination of apnoeas by administration of a CO_2-enriched gas mixture and addition of dead space (22). This has, however not translated into common clinical practice. Supplemental oxygen has also been used in ICSA (23). Adaptive servo ventilation (ASV) might be a reasonable treatment strategy in selected cases

Cheyne-Stokes breathing-central sleep apnoea (CSB-CSA)

CSB pattern's association with congestive heart failure is well known. Javaheri et al., MacDonald et al. and Oldenburg et al. have all reported on the high prevalence of CSB-CSA in stable heart failure patients (24-26). It is commonly associated with left ventricular systolic dysfunction but has also been described with diastolic dysfunction (27). CBS pattern is also seen in some neurological disorders such as cerebrovascular diseases and some neurodegenerative conditions. In heart failure associated CSB patients might report disturbed sleep. The majority of patients do not complain of subjective daytime sleepiness. Reduced survival rates in patients with heart failure and CSB pattern highlights its importance (28,29). Transplant free survival in patients with heart failure and CSB-CSA was significantly reduced compared to those without CSB-CSA independent of CPAP use (28,30).

Patients with CSB-CSA have a long circulation time. They tend to have relative daytime hypocapnia and the sleeping $PaCO_2$-AT difference is small. The ventilatory drive is high due to a high sympathetic tone and stimulation of respiration from pulmonary congestion. The supine position which worsens pulmonary congestion has been noted to increase the incidence of CSA. Polysomnographic studies demonstrate the typical crescendo-decrescendo morphology. There is a long delay in the nadir in the oxygen saturation tracing after event termination due to a prolonged circulation time. The typical CSB cycle time is long at 60-90 seconds. Risk factors for CSB-CSA include male gender, an older age, atrial fibrillation and daytime hypocapnia.

As with ICSA respiratory stimulants, hypnotics, oxygen therapy and PAP treatments have been tried in the treatment of CSB-CSA (23,31-33). Optimisation of the medical treatment of heart failure is the logical first step in the treatment of CSB-CSA. Respiratory stimulants such as theophylline and acetazolamide are not commonly used. Hypnotics are not in common use either. Krachman et al. compared oxygen therapy to CPAP and observed that both oxygen therapy and CPAP were equally effective at reducing the apnoea hypopnea index (AHI) in patients with heart failure and CSB (34). CPAP therapy has been noted to improve cardiac function (ejection fraction) reduce sympathetic activity and improve sleep continuity (35-37). However, it has not yet been fully shown to confer a survival benefit. The Canadian Continuous Positive Airway Pressure for Patients with CSA and Heart Failure (CANPAP) trial failed to show a survival benefit (38). The study also showed that only 50% of the patients responded to CPAP therapy after a 3-month period. A post hoc analysis of the responders showed improvement in survival and ejection fraction compared to controls. BiPAP with a backup rate has been shown to improve breathing in patient with CSB-CSA (25,39). In the past decade ASV has emerged as a promising treatment option for patients with CSB-CSA (25,40,41). It has been shown to be effective at reducing the AHI, oxyhaemoglobin desaturation index and at improving sleep quality. Some recent randomised controlled trials have also shown an improvement in cardiac function with ASV use. Kourouklis et al. treated nine patients with NYHA class II-III heart failure and at 6 months demonstrated significant improvement in left ventricular end diastolic volume and left ventricular ejection fraction compared to baseline parameters (42). Koyama et al. studied ten patients with heart failure who had CSA treated with ASV and compared to nine on conventional treatment (43). There was no improvement in echocardiographic parameters or plasma brain natriuretic peptide (BNP) levels in the conventional treatment group.

In those who used ASV, improvements were noted in left ventricular ejection fraction, left ventricular and systolic volume, and BNP. These researches also demonstrated that cardiac sympathetic nerve activity is associated with the severity of CSA.

Yoshihisa *et al.* randomised 36 patients with heart failure and preserved ejection and predominantly CSA to receive treatment with ASV plus conventional pharmacotherapy or pharmacotherapy alone (44). After 6 months of follow-up NYHA class, BNP, and diastolic function improved in the ASV group. High sensitivity troponin T was unchanged. Promisingly the event-free survival was higher in the ASV treated group compared to those receiving pharmacotherapy alone. Kasai *et al.* studied patients with moderate to severe CSB-CSA who had been on CPAP for 3 months but had failed to suppress AHI to below 15/hour (45). They then randomised them into a CPAP group and an ASV group and re-evaluated them after a further 3 months. Patients were assessed by echocardiography, polysomnography, plasma BNP, arterial blood gases, 24-hour urinary noradrenaline excretion, a 6-minute walk distance (6MWD) and the 36-item short form survey (SF36) quality of life tool at baseline and after 3 months. Of 12 patients completed the ASV arm and 11 patients completed the CPAP arm. AHI was significantly lower in the ASV group. Device compliance was better in the ASV group. Plasma BNP, LVEF, mitral regurgitation area and left ventricular end-systolic diameter were better with ASV compared to CPAP. Urinary noradrenaline excretion and 6MWD were not significantly different within group comparisons. Quality of life score as determined by the SF-36 was also better with ASV. In another randomised controlled trial comparing standard pharmacotherapy to pharmacotherapy plus ASV in patients with systolic heart failure and CSA/HCSB, Hetland and co-workers demonstrated that after 3 months of treatment, ASV patients had significant increases in LVEF, improved NYHA class, and longer 6MWD as well as a possible trend toward reduced congestive heart failure mortality but this was only at 3 months and the numbers were small (30 patients) (46). In another randomised controlled trial comparing standard pharmacotherapy with pharmacotherapy plus ASV in patients with systolic heart failure and sleep disordered breathing that was either OSA and/or CSA, LVEF increased slightly after 12 weeks in both groups by intention-to-treat and per-protocol analysis, whereas N-terminal pro-BNP remained significantly higher in the control group (47). The outcome of the ongoing multinational and multicentre trial on The Effects of Adaptive Servo Ventilation on Survival and Frequency of Cardiovascular (ADVENT-HF) will be eagerly awaited. Another multinational and multicentre randomised study looking at the treatment of sleep-disordered breathing with predominant CSA with adoptive servo-ventilation in patients with chronic heart failure (the SERVE-HF study) which is also currently in progress is hoped to provide data on the effect of treatment with ASV on morbidity and mortality as well as cost effectiveness of this treatment.

Complex SA

Complex SA is identified by emergence or persistence of central apnoea upon exposure to CPAP treatment. PAP treatments eliminate the upper airway obstruction but do not correct the ventilatory control instability or the sleep state instability (48). Relieving the upper airway resistance might result in lower $PaCO_2$. The reasons for the emergence of central apnoeas remain obscure. It is likely that a high loop gain could be responsible for its persistence. Sleep disturbance due to failure to acclimatise to CPAP might also cause frequent arousals leading to CSA. CPAP induced expansion of lung volumes might lead to prolonged expiration to the point of central apnoea in some patients. The prevalence of complex SA has been reported to be between 7% and 20% of patients put on CPAP therapy with a male preponderance being also noted (24,48-50). Symptoms include persistence of day time sleepiness despite CPAP treatment and disturbed non refreshing sleep. Polysomnographic studies usually reveal obstruction or mixed apnoeas during the diagnostic studies. During CPAP titration the AHI remains elevated. A number of studies have shown that complex SA will spontaneously resolve with chronic CPAP use. If a patient is tolerating CPAP well they might therefore be no need to change treatment. BiPAB with a backup rate can be an effective alternative (51). ASV can be used for those who do not improve. A number of studies have demonstrated its effectiveness in patients with complex SA (51-53).

High altitude periodic breathing

High altitude breathing occurs following recent ascent to altitude of at least 4,000 metres. It is a normal physiological adaptation to hypoxia at hypobaric altitudes. Hypoxia at altitude increases ventilatory drive which then leads to periodic breathing (54). Polysomnographic studies demonstrate recurrent central apnoeas primarily during NREM sleep at a frequency of >5/hour. The cycle length

Box 2 Primary sleep apnoea of infancy—diagnostic criteria
Apnoea of prematurity
One of the following is recorded in an infant <37 weeks conceptional age:
Prolonged central respiratory pauses of The mechanisms by which oxygen therapy works is not entirely clear, but it is hypothesized that is has an effect on reducing peripheral chemoreceptor sensitivity to carbon dioxide which allows $PaCO_2$ to elevate above the apnoeic threshold and reduces likelihood of instability ≥20 seconds in duration
Shorter-duration events include obstructive or mixed respiratory patterns and are associated with a significant physiologic compromise, including decrease in heart rate, hypoxia, clinical symptoms or the need for nursing intervention
Apnoea of infancy
One of the following is recorded in an infant of conceptional age ≥37 weeks
Prolonged central respiratory pauses of ≥20 seconds in duration
Shorter duration events including obstructive or mixed respiratory patterns and are associated with bradycardia, cyanosis, pallor, or marked hypotonia
For either diagnosis, the disorder is not better explained by another current sleep disorder, medical or neurologic disorder, or medication
From the American Academy of Sleep Medicine: ICSD-2 2005 (1). ICSD, International Classification of Sleep Disorders.

should be 12-34 seconds. Symptoms include disturbed poor quality sleep or a sense of suffocation. Most people will gradually acclimatize to altitude. Sleep disturbance can be helped by benzodiazepines. Nickol *et al.* demonstrated that at high altitude temazepam is effective in reducing periodic breathing, and is safe to use, without adverse effect upon next day performance (55). Acetazolamide also helps sleep disturbances (56). In a randomised three way cross over study by Hackett *et al.* Acetazolamide was noted to be superior to almitrine at ameliorating periodic breathing (54). Almitrine and acetazolamide both increased saturations during sleep but it was only the acetazolamide that decreased periodic breathing. An earlier randomised double blind placebo controlled study by Fischer *et al.* demonstrated that both theophylline and acetazolamide improved sleep disordered breathing and reduced oxhaemoglobin desaturation during sleep, with acetazolamide significantly improving basal oxyhaemoglobin saturation during sleep (57). Tanner *et al.* performed a randomized, double-blind trial of temazepam and acetazolamide at an altitude of 3,540 meters on 34 healthy trekkers with self-reports of high-altitude sleep disturbance (56). They concluded that treatment of high-altitude sleep disturbance with temazepam is associated with increased subjective sleep quality compared to acetazolamide.

CSA due to medication (opiate/narcotic induced CSA)

Drugs in common use for pain control include morphine,

fentanyl and methadone. Patients on high doses of these medications may have a slow sleeping respiratory rate. They may develop long obstructive apnoeas. Ataxic breathing is commonly observed with variation in respiratory rate and tidal volume. Relatively few arousals are noted and stage N3 can be increased. Periodic breathing and intermittent central apnoeas are seen and these may even occur in stage N3. Treatment emergent central apnoeas are also common even if the diagnostic study might have shown mainly obstructive events. Reduction in opiate dose is a logical first step if this were possible. Patients with mostly obstructive events respond to CPAP but then central apnoeas may emerge or persist (58). Javaheri *et al.* reported on ASV being effective treatment for both central and obstructive apnoea in opiate induced complex SA (40). It is essential to increase EPAP sufficiently to eliminate upper airway obstructive events in these situations.

Primary sleep apnoea of infancy

Primary sleep apnoea of infancy is due to immaturity of the respiratory control system. It is deemed pathological only if the duration of apnoea is greater than 20 seconds or if the events are associated with desaturation or other physiological compromise. The ICSD-2 diagnostic criteria are outlined in *Box 2*. Therapy is often mostly just supportive management. The primary pharmacologic agents used to treat apnoea of prematurity are caffeine and theophylline (59).

Conclusions

Of the CSA syndromes CSB in heart failure is the most common and most important. There is mounting evidence that its presence can adversely affect outcome in affected individuals. In the past decade ASV has emerged as a promising treatment modality which might improve cardiac function, improve sleep quality and possibly confer a survival benefit. Outcomes of the studies looking into the beneficial effects of its long term use are eagerly awaited. Complex SA has also been increasingly recognised with the widespread use of CPAP for OSA. With the more liberal administration of strong opiates to treat pain syndromes central apnoea due to drug or substance is also now being increasingly encountered. A back to basics physiological approach helps in the understanding of central apnoea syndromes. Further physiological and clinical research is still required into this field.

Acknowledgements

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Chronic hypoventilation syndromes and sleep-related hypoventilation

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Abstract: Chronic hypoventilation affects patients with disorders on any level of the respiratory system. The generation of respiratory impulses can be impaired in congenital disorders, such as central congenital alveolar hypoventilation, in alterations of the brain stem or complex diseases like obesity hypoventilation. The translation of the impulses via spinal cord and nerves to the respiratory muscles can be impaired in neurological diseases. Thoraco-skeletal or muscular diseases may inhibit the execution of the impulses. All hypoventilation disorders are characterized by a reduction of the minute ventilation with an increase of daytime hypercapnia. As sleep reduces minute ventilation substantially in healthy persons and much more pronounced in patients with underlying thoraco-pulmonary diseases, hypoventilation manifests firstly during sleep. Therefore, sleep related hypoventilation may be an early stage of chronic hypoventilation disorders. After treatment of any prevailing underlying disease, symptomatic therapy with non-invasive ventilation (NIV) is required. The adaptation of the treatment should be performed under close medical supervision. Pressure support algorithms have become most frequently used. The most recent devices automatically apply pressure support and vary inspiratory and expiratory pressures and breathing frequency in order to stabilize upper airways, normalize ventilation, achieve best synchronicity between patient and device and aim at optimizing patients' adherence.

Keywords: Chronic hypoventilation syndromes; sleep related hypoventilation; hypercapnic respiratory failure; pathophysiology; obesity hypoventilation syndrome (OHS)

Introduction

Under physiological conditions, alveolar ventilation is closely adapted to metabolism. The minute ventilation is regulated according to the prevailing carbon dioxide production so that the arterial carbon dioxide partial pressure ($PaCO_2$) is adjusted to values between 35-45 mmHg. Increases (hyperventilation) or decreases (hypoventilation) of the minute ventilation lead to excess elimination or accumulation of carbon dioxide,

respectively, and destabilize the acid-base balance. However, consecutive increases of the pH value (respiratory alkalosis) or decreases (respiratory acidosis) are only measured during acute changes of ventilation. In contrast, if hyper- or hypoventilation continues for hours, the pH level is stabilized by variations of the renal bicarbonate (HCO_3^-) excretion (1). Therefore, chronic hypoventilation syndromes are characterized by:

- Diminishment of the minute ventilation, i.e.,

reductions of the tidal volume or breathing frequency;
- Elevation of the $PaCO_2$ >45 mmHg;
- pH level in a normal range and an increase of HCO_3^- (metabolic compensation).

Chronic hypoventilation is also called hypercapnic respiratory failure or type II respiratory failure. The diminution of minute ventilation can generate from all levels of the respiratory system including insufficient respiratory drive (brain stem), impaired transmission of the breathing impulses (spinal cord, peripheral nerve), and morphological or functional abnormalities of the musculo-skeletal system of the thorax. Failure of the respiratory regulation on the one hand or execution of the impulses on the other hand represent different facets of the pathophysiology which can be described as "won't breathe" and "can't breathe" (2). Congenital central alveolar hypoventilation and opioid use represent examples of central disturbances, while amyotrophic lateral sclerosis (ALS), phrenic nerve palsy, muscle dystrophies, idiopathic sclerosis are examples of insufficient execution of ventilation. However, the obesity hypoventilation syndrome (OHS) combines components of both pathomechanisms. Chronic hypoventilation with daytime hypercapnia and sleep-related hypoventilation (SRH) do not differ substantially. Actually, SRH seems to represent an early stage of chronic hypoventilation. Nevertheless, this hypothesis has to be confirmed and the number of patients with advancing severity has to be evaluated in future research.

The most recent edition of the International Classification of Sleep Disorders (3) describes six subtypes of hypoventilation disorders in the chapter of sleep related breathing disorders:
- OHS;
- Congenital central alveolar hypoventilation syndrome (CCHS);
- Late-onset central hypoventilation with hypothalamic dysfunction;
- Idiopathic central alveolar hypoventilation;
- Sleep related hypoventilation due to a medication or substance;
- Sleep related hypoventilation due to a medical disorder.

The term CCHS replaces the name "Ondine's curse". CCHS requires evidence of a mutation of the PHOX2B-gene leading to a diffuse imbalance of the autonomic system. Differences in the mutation discriminate congenital from *de novo* development (4). Despite its congenital character, the disease may manifest in adulthood in some cases (3,5). Late-onset phenotypes may present with respiratory failure after general anesthesia, severe respiratory illness or respiratory depressants (6). However, in most cases hypoventilation begins during childhood and is more pronounced during sleep. The failure of central respiratory drive may be associated with respiratory rest during sleep. CCHS may be accompanied with other phenomena like Hirschsprung's disease, cardiac arrhythmia, and tumors (6,7).

Idiopathic central alveolar hypoventilation is diagnosed if diseases of lung parenchyma, airways, pulmonary vessels, chest volume or neuromuscular diseases (NMD), drug treatment, obesity or congenital hypoventilation can be excluded (3,5). Several questions on idiopathic central alveolar hypoventilation are unresolved. The pathophysiology is unclear although an impairment of the hypercapnic and hypoxic chemoresponsiveness and respiratory drive has been discussed.

Late-onset central hypoventilation with hypothalamic dysfunction is a disorder of the central control of ventilation (3). It can be diagnosed if sleep related hypoventilation develops after the first years of life. The patients present with obesity, endocrine abnormalities of hypothalamic origin, severe emotional or behavioral disturbances or a tumor of neural origin (two of these four findings are required). Moreover, mutations of the PHOX2B-gene and other disorders explaining hypoventilation have to be excluded. Although the disease is associated with hyperphagia, hypoventilation persists even if patients lose weight. Diabetes insipidus, inappropriate antidiuretic hormone hypersecretion, precocious puberty, hypogonadism, hyperprolactinemia, hyperthyroidism and decreased growth hormone secretion are associated endocrine dysfunctions (3,5).

In contrast to these rare disorders, the OHS and chronic hypoventilation due to medical disorders or pharmaceutical influences represent the huge majority of chronic and SRH. OHS patients are characterized by obesity (BMI >30 kg/m²) and wakefulness hypercapnia. Hypoventilation in OHS cannot primarily be explained by other thoraco-pulmonary, neuromuscular or idiopathic diseases or pharmaceutical influences (3,5,8,9) (*Table 1*).

Sleep related hypoventilation due to a medical disorder is diagnosed in patients with underlying diseases of the lung parenchyma or the airways, the pulmonary vessels or neurological or musculo-skeletal disorders. In addition, SRH can be induced by drugs which depress ventilatory drive or impair muscle function. Long-acting narcotics, anesthetics, sedatives and muscle relaxants and also alcohol have been discussed (3,5). Chronic opioid intake may be associated with central apnoeas, atactic respiration but also

Table 1 Characteristics of the obesity hypoventilation syndrome (OHS)

Definition criteria	Description
Chronic hypoventilation	Hypercapnia in wakefulness and sleep (PaCO$_2$ ≥45 mmHg or 6 kPa)
Obesity	Body mass index (BMI) ≥30 kg/m²
Absence of other causes of hypercapnia	Diseases of the lung parenchyma, airways, pulmonary vessels
	Neurological, musculo-skeletal or other idiopathic disorders
	Congenital central alveolar hypoventilation syndrome (CCHS)
	Medication or drugs
Sleep-related breathing disorders	Obstructive sleep apnea (OSA) with or without coexisting sleep-related hypoventilation in 90% of patients
	Non-obstructive (apnea-hypopnea index <5 events/hours) sleep-related hypoventilation in about 10%

sustained hypoxemia and hypoventilation (10).

Diagnosis of chronic and sleep related hypoventilation

Due to the broad variety of underlying diseases and pathophysiological mechanisms, there are no single typical clinical signs or symptoms which confidentially indicate or predict chronic or sleep related hypoventilation. Thus, a comprehensive clinical assessment, including a detailed history on sleep quality, morning symptoms, daytime fatigue or dyspnoea on exertion and a careful examination are crucial. Firstly, impaired alveolar ventilation becomes evident during sleep or exertion. Sleep is associated with a reduction of the minute ventilation even in healthy persons, while physical stress increases CO$_2$ production. Hypoventilation during sleep may be associated with poor sleep quality, excessive daytime sleepiness and morning headaches. However, a relevant portion of patients reports no or only minor complaints. Typical clinical symptoms include reduced exercise capacity and dyspnoea. However, there are huge interindividual differences in clinical findings also depending on the underlying disease (6,11).

Chronic hypercapnic respiratory failure and hypoventilation during exertion can easily be diagnosed by arterial or capillary blood gas analysis during wakefulness. However, monitoring of respiration and carbon dioxide levels during sleep are needed to establish the diagnosis of SRH. Polysomnography (PSG) reveals the gold standard of investigating sleep and respiration. It is the only technique which allows to differentiate sleep and wakefulness and to diagnose electroencephalographic arousals and their relation to breathing disturbances. Sleep-wake transitions

and arousals substantially influence respiration, leading to central breathing disturbances and propagation of periodic breathing (12-14). Thus, PSG is crucially important to precisely define the disease in individual patients and understand the underlying pathophysiology. Moreover, optimal therapy of chronic hypoventilation should not only focus on improvement of oxygen saturation and normalization of hypercapnia, but also in stabilization of the sleep profile. Therefore, we recommend PSG in the diagnostical work up of patients with chronic hypoventilation and in the follow-up of patients with persisting fatigue, sleepiness and morning headache under treatment. Nevertheless, if PSG is not available or cannot be performed due to comorbidities or complicated circumstances, multi-channel respiratory studies may suffice (15). When combined with actigraphy, they may also allow to separate sleep from wake periods (16).

Different invasive and non-invasive techniques are available to measure the carbon dioxide level. Arterial blood samples or samples from arterialized ear lobe represent the state of the art techniques for assessment of the PCO$_2$. However, blood sampling during the night disrupts patients' sleep, which may be associated with hyperventilation. Moreover, as a snapshot, single samples may not reflect the ventilator status of the whole night (17). Monitoring of the end-tidal CO$_2$ (PetCO$_2$) and of the transcutaneous CO$_2$ (PtcCO$_2$) allow for non-invasive and continuous measurement. PetCO$_2$ is known to be influenced by nasal congestion and secretion. Moreover, it may substantially be limited by oxygen insufflation, non-invasive ventilation (NIV) and mask leaks (18,19). PtcCO$_2$ allows for reliable and continuous measurement of the changes of the parameter. PtcCO$_2$ correlates with PaCO$_2$

although absolute figures often differ substantially. Actually, $PtcCO_2$ represents a different parameter as it is influenced by the metabolism of the skin cells and the heating of the skin. Thus, $PtcCO_2$ is systematically higher than $PaCO_2$, which has to be considered when interpreting the results. In addition, there might be a shift of the $PtcCO_2$ during long term measurements, although this problem seems to be less relevant with modern devices (17,20).

Numerous definitions of SRH have been introduced; they impair the comparison of studies and may have impact on clinical decisions. Previous definitions included increases of the $PaCO_2$ >50 mmHg for >5% of measuring time or 10 mmHg rise or peak $PtcCO_2$ >6.5 kPa (49 mmHg) (15). The most recent revision of the American Academy of Sleep Medicine (AASM) scoring criteria suggest to score SRH in case of:

- PCO_2 >55 mmHg for ≥10 minutes during sleep or;
- Increases of the PCO_2 ≥10 mmHg as compared to awake supine value up to a level of >50 mmHg for ≥10 minutes (21);
- In children, hypoventilation is scored if the $PaCO_2$ or surrogate parameter increase >50 mmHg for >25% of total sleep time (21).

Hypoventilation is usually associated with a long term oxygen desaturation so that a decreased of the oxygen saturation (SaO_2) <90% for >5 minutes with a nadir of ≤85% may also indicate hypercapnia and should urge to further diagnostical work up. Finally, as mentioned above, elevated levels of HCO_3^- after awakening suggest sleep hypoventilation even if the $PaCO_2$ is within the normal range during wakefulness.

Identification of patients at risk for hypercapnic respiratory failure

An inversed breathing pattern in supine position may indicate a relevant deterioration of ventilatory muscle capacity in NMD. The change of the forced vital capacity (FVC) from erect to supine position may deliver additional information: while FVC decreases approximately by 8-10% from upright to supine position in healthy subjects, Allen *et al.* showed that a decrease >25% indicates an impaired diaphragmatic function (22). Ragette *et al.* found a correlation between inspiratory vital capacity (IVC) with respiratory muscle function and CO_2 elimination in NMD during day and night. Onset of sleep disordered breathing was noticed with IVC <60%, while a figure below 40% was associated with continuous hypoventilation during sleep, respiratory

failure both during sleep and wakefulness was likely below 25% (23). The sniff nasal pressure was superior to vital capacity (VC) in predicting reduced respiratory muscle strength in ALS without significant bulbar involvement (24). In addition, children with NMD exhibit significantly more often sleep related hypoventilation when they also suffer from scoliosis (25). Lung function parameters indicating a high respiratory load and low muscle capacity are major predictors of daytime hypercapnia in COPD (26). Forced expiratory volume in one second (FEV_1) correlates with chronic hypercapnia in most studies. Montes de Oca and Celli studied 33 severe COPD patients, including 14 with daytime hypercapnia, and 20 controls. The likelihood of hypercapnia increased substantially if FEV_1 was <0.5 liters (27). Hypercapnia was more probable in patients with a FEV_1 <40% and in hyperinflation as demonstrated by Rodríguez-Roisin *et al.* (28). and Saure *et al.* (29).

Increased levels of HCO_3^- may indicate chronic or intermittent hypoventilation. It has been shown, that an elevated HCO_3^- level sufficiently predicts hypercapnia in obese subjects (30). The careful examination of the relation between $PaCO_2$ and body-mass-index (BMI) may prevent underdiagnoses of OHS. Bülbül *et al.* found a ratio below 1.5 to be strongly predictive of the disease (31). In addition, oxygen saturation during sleep or wakefulness may also serve as an indicator of chronic hypercapnia. Basoglu *et al.* found an independent association of hypercapnia with reduced daytime SaO_2 in OHS as compared to matched patients with obstructive sleep apnoea (OSA) (32). Parameters of lung function may reveal a restrictive pattern in morbidly obese patients including reduced VC and FEV_1 which may be amplified in patients with OHS (33).

Pathophysiology of chronic hypoventilation

Although the reduction of the minute volume is the common characteristic of all chronic hypoventilation disorders, the underlying pathomechanisms differ substantially between the entities and can be complex in individual cases (exemplary shown for OHS in *Figure 1*).

Respiratory mechanics importantly contribute to chronic hypoventilation in many conditions. Inspiratory muscle strength diminishes during the course of NMD with diaphragmatic involvement. Scoliosis and thoracic hyperkyphosis—idiopathic or secondary to NMD—may impair the diaphragm capacity and contribute to thoraco-pulmonary restriction.
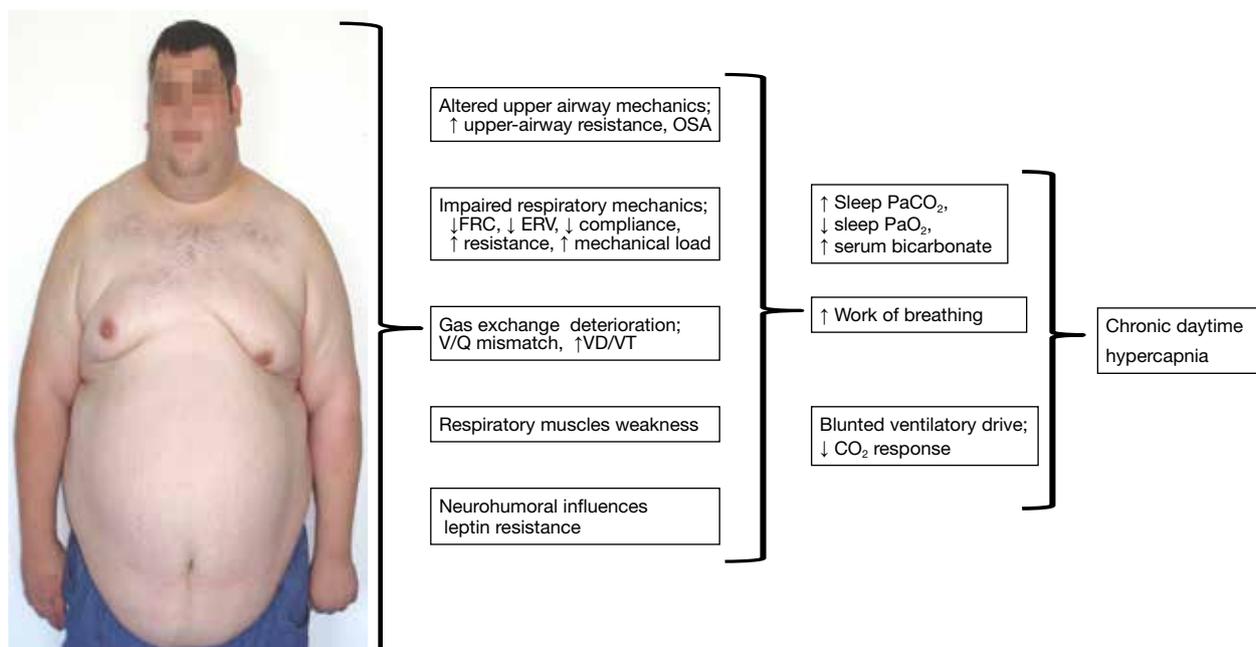
Figure 1 Possible mechanisms by which obesity can lead to chronic daytime hypercapnia. CO_2, carbon dioxide; ERV, exspiratory reserve volume; FRC, functional residual capacity; OSA, obstructive sleep apnea; $PaCO_2$, arterial carbon dioxide partial pressure; PaO_2, arterial oxygen partial pressure; Q, perfusion; V, ventilation; VD, dead space volume; VT, tidal volume.

Obesity adds additional mass load to the respiratory system and reduces lung volume, especially when severe and predominantly centrally distributed (34). It increases the resistance of the upper and lower airways and reduces the compliance of the respiratory system. Sharp *et al.* showed in the 1960s that the compliance is reduced by 60% in OHS patients as compared to non-obese patients and by 20% as compared to normocapnic obese patients (35). The small airways tend to collapse at low tidal volumes leading to trapping of the air and increasing intrinsic end-expiratory pressure (PEEPi) (36). All these factors elevate the work of breathing in OHS patients, both in upright and supine position (37). On the other hand, obesity reduces the expiratory reserve volume, leading to ventilation perfusion mismatch and abnormalities of the gas exchange (38).

Similar effects on PEEPi limit the VC in COPD patients with severe hyperinflation. The flattening of the diaphragm impairs its mechanical properties and increases the work of breathing (39).

In addition to alterations of the thoraco-pulmonary mechanics, the ventilatory control system contributes substantially to chronic hypercapnic failure and sleep related hypoventilation. While respiration is primarily driven by the carbon dioxide level in healthy subjects, the response

to hypercapnia is usually altered in chronic hypercapnia. Radwan *et al.* compared patients with overlap of COPD and OSA with patients with OSA alone. Plasma bicarbonate concentration was significantly elevated in overlap patients, indicating chronic or long term nocturnal hypoventilation. Moreover, the hypercapnic ventilatory response was reduced in overlap patients, while it was normal in awake OSA patients (40).

Hypoxic and hypercapnic ventilatory response is also blunted in OHS patients as compared to normocapnic OSA patients (41). There is no evidence of impaired chemoresponsiveness in first degree relatives of OHS patients making an inherited condition improbable (42). However, the reduced chemoresponsiveness discriminates OHS patients from normocapnic obese individuals and OSA patients.

An impairment of the hypercapnic ventilatory response also contributes to the pathophysiology of NMD (15). This hypothesis is supported by findings of Nickol *et al.* in patients with hypercapnic respiratory failure due to restrictive thoracic diseases (NMD or chest wall disorders). Nocturnal NIV effectively controlled daytime $PaCO_2$, although neither muscle strength, nor lung function or respiratory compliance significantly increased. Therefore, the authors

concluded that the normalization of the chemosensitivity is the principal mechanism improving gas exchange under NIV (43).

The impairment of the central ventilatory drive may be the exclusive pathophysiological factor in rare cases, such as CCHS. They present with diminished minute ventilation both, during wakefulness and sleep which is most pronounced in non-rapid-eye-movement (NREM) sleep (44).

Leptin is a protein specifically produced by adipose tissue. *Leptin* crosses the blood-brain barrier and interacts with specific receptors in various areas of the brain and—among other effects—stimulates ventilation (45). Its contribution to the pathophysiology of OHS has intensively been discussed in recent years. The serum *leptin* concentration is elevated, associated with increased ventilation in obese individuals. This is thought to compensate for the increased CO_2 production by excess body-mass (33). Shimura *et al.* compared circulating levels of *leptin* in OSA patients with and without hypercapnia. Serum *leptin* correlated with the BMI and was the only predictor for hypercapnia (46). However, *leptin* failed to adequately stimulate ventilation in hypercapnic individuals, which has been interpreted as a central *leptin* resistance contributing to the pathophysiology of OHS (11).

Obstructions of the upper airways additionally stress the ventilatory system and may impede CO_2 elimination especially during sleep. Obstructive apnoeas and hypopnoeas are associated with transient episodes of acute hypercapnia. While eucapnic OSA patients hyperventilate between obstructive episodes and therefore eliminate the accumulated CO_2 (47), the duration of the interval and increase of the minute ventilation may not allow for normalization of CO_2 in patients with sleep related hypoventilation (48). Computer models suggest, that CO_2 accumulates over the long term, when apnoea episodes become more than 3 times longer than the hyperventilation period between them (15). Obstructions of the upper airways may also be involved in patients with neuro-muscular diseases. The muscle of the upper airways may directly be involved if bulbar nerves and depending muscles are affected. The function of the upper airway muscles can be impaired in myopathic disorders or under pharmaceutical influences (long-term use of corticosteroids). Fat deposition and fluid retention may narrow the diameter of the upper airways. Peripheral edema and fluid overload frequently occur in patients with right heart failure in COPD or OHS. The fluid may shift from the lower limbs to the upper body

components during recumbency. Redolfi *et al.* showed in non-obese men that the severity of OSA strongly correlates with the reduction of leg fluid volume and concomitant increase in neck circumference (49). In addition to the increased load to the uppper airways, they may collapse in central breathing disturbances (50). The capacity of the muscles may be insufficient to compensate for the additional load and the increased resistance in patients with NMD or thoraco-skeletal disorders.

As mentioned before, pharmaceutical therapy can also negatively impact ventilation. Steroid myopathy is an adverse effect of high dose, long-term use of systemic corticosteroids. Although corticosteroid-induced muscle atrophy affects predominantly type II b muscle fibers (51), additional effects on accessory respiratory muscles have to be discussed. Moreover, the long-term systemic treatment with glucocorticosteroids is a risk factor for the development and worsening of osteoporosis. The prevalence of osteoporosis in COPD varies between 9-69% and exceeds prevalence in healthy subjects (52). However, the causal relationship between corticosteroids and osteoporosis in COPD has not undoubtedly been demonstrated. Vertebral fractures due to osteoporotic sinterings and consecutive reductions of the weight diminish the efficiency of respiratory muscles. Recently, Watanabe *et al.* found an association between osteoporosis on the one hand and deterioration of pulmonary function on the other in Japanese male patients with COPD (53).

Opioids are often prescribed for chronic pain or palliation of dyspnoea in patients with severe, symptomatic lung disorders. They influence ventilation by blunting the hypercapnic ventilatory response, reducing breathing frequency, inducing the collapse of the upper airways and diminishing the activity of peripheral muscles (54). In addition to short term apnoeas and hypopnoeas, sustained hypoxia during sleep may delay arousals and increase the arousal threshold in NMD, COPD and OHS (55).

Influence of sleep on ventilation

Hypercapnia manifests during sleep prior to wakefulness as a consequence of physiological and pathophysiological changes of ventilation. The minute ventilation decreases from wakefulness to NREM sleep and further to rapid-eye-movement (REM) sleep by about 15% in healthy subjects (56).

The reduction of the minute ventilation during sleep is predominantly due to a lower tidal volume, which is not fully compensated by an increase of breathing frequency. The
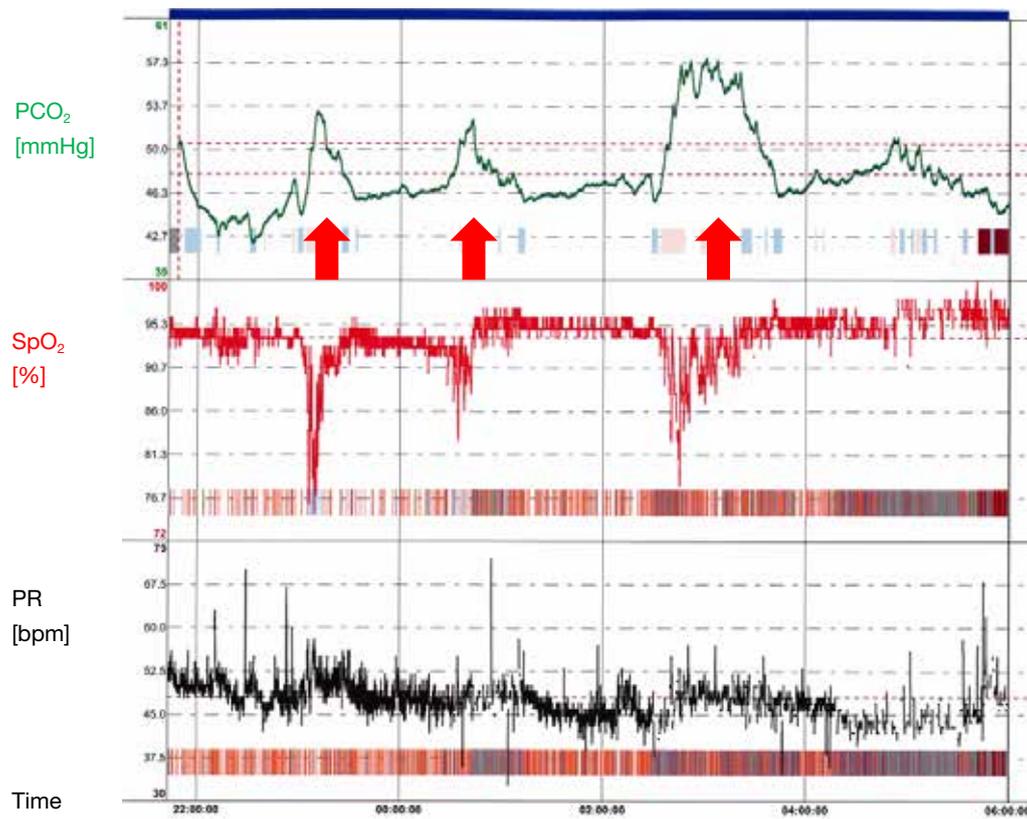
Figure 2 Nocturnal transcutaneous CO_2 monitoring shows three sustained episodes of elevated PCO_2 levels (red arrows) and corresponding decreases in SpO_2. Polysomnography (not shown in this figure) revealed rapid eye movement (REM) sleep during these episodes. PCO_2, carbon dioxide partial pressure; SpO_2, oxygen saturation; PR, pulse rate.

underlying pathophysiological mechanisms are complex: the tone of the thoracic muscles is reduced during sleep, reaching its lowest level during REM. The muscle relaxation also increases the upper airway resistance (50) and thus predisposes to upper airway obstruction. As discussed above, obstructive breathing disturbances account for further CO_2 loading in obese patients and patients with NMD.

The muscle atonia during REM sleep affects primarily the accessory breathing muscles, whereas diaphragm contraction is saved. However, lung hyperinflation in COPD reduces the efficiency of the diaphragm, leading to a reduction of the tidal volume and the minute ventilation (9,57). It may also be reduced in diseases, in which accessory breathing muscles contribute substantially to ventilation.

In addition, hypoxic and hypercapnic ventilatory responses are also blunted during REM sleep, leading to insufficient reactions to changes of the blood gases. Respiratory derailments during REM as well as NREM sleep trigger arousals, resulting in sleep fragmentation and diminished sleep efficacy. Thus, taking all these aspects together, sleep related hypoventilation manifests during REM sleep at first (*Figure 2*). PSG analyses in NMD showed a significant reduction of the REM proportion which might be regarded as protective mechanism to respiratory problems during REM sleep (58).

In clinical practice, it is not uncommon, that patients present with both, different entities of sleep related hypoventilation and other breathing disturbances during sleep. Ninety percent of OHS patients exhibit OSA (11). Nocturnal hypercapnia is more prevalent in COPD patients if they suffer from comorbid OSA (59). Their coexistence is referred to as the overlap syndrome and may be associated with pulmonary hypertension and right heart failure (60). Breathing disturbances in patients with Duchenne muscular dystrophy may begin with OSA (61), followed by sleep related hypoventilation when diaphragmatic weakness

becomes critical and end with chronic hypoventilation. Several investigators have focused on breathing disturbances under chronic opioid use in recent years. Rose *et al.* showed chronic hypercapnic failure in patients with chronic pain and long term opioid therapy. In addition to hypoventilation, up to 50% of the patients presented with severe sleep apnoea, predominantly of central origin (62). CCHS is characterized by disturbed central chemical respiratory drive. However, central apnoeas and hypopnoeas have been described in PSG-studies of affected patients so that CCHS may present as predominant central sleep apnoea in individual cases (63).

Treatment of chronic hypoventilation

It is a general principle in medicine to treat any causative factors if possible. In terms of chronic hypoventilation this includes the cessation or reduction of drugs affecting breathing regulation, nerval transmission or muscular function. Opioids, benzodiazepine and other psychotropic drugs have to be reviewed critically. The muscles should be unloaded or their efficiency improved. Therefore, weight reduction, stabilization of vertebral fractures, orthopedic and surgical methods in kyphoscoliosis should be discussed. Electrical stimulation of the diaphragm can improve ventilation in individual cases (64,65).

However, causal treatment options will not suffice for the huge majority of patients with chronic or sleep related hypoventilation so that symptomatic therapy with mechanical ventilation becomes necessary. During the poliomyelitis pandemics in the 20th century, negative pressure ventilators—popularly named iron lungs, or steel cocoons—saved the life of thousands of patients afflicted with respiratory muscle paralysis (66). Negative pressure ventilation has completely been replaced by devices, which non-invasively apply ventilatory support via a nasal or oral-nasal mask to the patient. NIV generates the tidal volume by a fixed difference between inspiratory and expiratory pressure (pressure support, pressure controlled ventilation) or a predefined volume (volume support, volume controlled ventilation).

Optimal treatment of patients with chronic hypoventilation with NIV crucially depends on the underlying pathophysiological mechanisms. Algorithms primarily focusing on pressure support are most frequently used. They allow to separately adapting inspiratory and expiratory pressure and back-up frequency. The expiratory positive airway pressure (EPAP) is titrated according to the level of

upper airway obstruction (12). It dilates the upper airways and therefore reduces the work of breathing to overcome upper airway resistance. In addition, the expiratory pressure stabilizes the small airways and may overcome the intrinsic peak, it increases air flow to atelectatic parts of the lungs and improves ventilation perfusion mismatch. The EPAP should be titrated under polygraphic or polysomnographic supervision, aiming at optimizing oxygen saturation, reducing apnoeas, hypopnoeas and flattening of the flow curve and respiration-related arousals. The improvement of sleep quality and the reduction of sleep-wake transitions avoid central breathing disturbances and stabilize ventilation (avoid periodic breathing) (12-14).

Tidal volume and breathing frequency influence the CO_2 elimination and therefore counterbalance chronic or intermittent hypoventilation. Therefore, the inspiratory positive airway pressure (IPAP) is not the primary target of titration but the difference between EPAP and IPAP (Δ IPAP—EPAP) which defines the tidal volume. When EPAP has been titrated, the Δ IPAP—EPAP (the pressure support) can be adapted aiming at normalization of $PaCO_2$ or $PtcCO_2$.

The back-up frequency eliminates any central apnoeas and overcomes periods of bradypnoea. If the back-up frequency is set above the spontaneous breathing rate, the patient is fully controlled ventilated. In terms of patients' compliance and synchronization, it is often reasonable to set the back-up frequency slightly below the spontaneous breathing frequency of the patient (67).

Taking these aspects together, the ventilator settings should be individually selected based on the underlying pathophysiological components (12):

- Airway obstruction can be addressed by increasing intraluminal expiratory pressure [CPAP, EPAP, bilevel in the spontaneous mode (BiPAP-S)];
- Reduced ventilatory drive (breathing frequency) can be counterbalanced by the application of mandatory breaths;
- Fixed or variable pressure support and mandatory breaths assure the necessary minute ventilation (12).

Controlled versus assisted NIV

Volume-controlled or pressure-controlled ventilation previously represented two extremes of NIV. While the former applied a fixed, predefined tidal volume, irrespective of the required inspiratory pressure level, the latter delivered a fixed pressure support, independent of the really

applied volume. It has been hypothesized that optimal ventilation depends on maximal unloading of the respiratory muscles. This would allow restoring energy reserves required for spontaneous respiration. Controlled ventilation strategies unload respiratory muscles most intensively, as the ventilator takes over the complete work of breathing (68). However, synchronicity of the patients' breathing rhythm with the ventilator may impact efficacy and tolerance. In addition, studies in animals and invasively ventilated intensive care patients have shown negative impact of controlled ventilation on muscle structure and function. In contrast, assisted ventilation allows the patient to spontaneously trigger pressure or volume support, which may improve synchronicity and may damage muscle fibers in less extent (69-71). In clinical practice, non-invasive pressure support ventilation has become the most favorite therapeutical approach. However, modern NIV devices work in hybrid modes: they apply a defined pressure support, a minimal preset tidal volume and minimal rate of mandatory breaths, combining the advantages and disadvantages of the algorithms.

Specific clinical situations may require variable ventilatory support, e.g., according to changing of the body position, sleep stage or patient's respiratory drive. The most recent algorithms of NIV devices allow for automatically varying the expiratory pressure to overcome upper airway obstruction and adjusting the pressure support in order to ensure a predetermined target: pressure support ventilation with target volume (distributed as: average volume assured pressure support, AVAPS) (72-74). Nevertheless, the physician has to supervise the adaptation process and evaluate the effect on carbon dioxide, sleep parameters and upper airway obstruction. The automatic algorithms with a volume assurance may allow for individualized therapy but have not shown to be superior in general.

NIV in specific situations

NIV is indicated in neuromuscular and restrictive disorders and OHS if patients present with daytime hypercapnia >45 mmHg. In addition, ventilator support may be introduced in neuromuscular or thoraco-skeletal disorders in symptomatic nocturnal hypoventilation even without daytime hypercapnia (15). Keeping in mind that symptoms of nocturnal hypoventilation are highly variable and non-specific, a careful patient examination is necessary at each follow-up consultation. Additional factors emphasizing NIV include comorbidities with upper airway obstruction or impaired peak cough flow. NIV is indicated

in COPD patients if they present with chronic daytime hypercapnia ≥50 mmHg or a $PaCO_2$ of 46-50 mmHg associated with ≥2 hospitalizations within the last 12 months due to hypercapnic respiratory failure (75).

Volume-targeted algorithms may be favorable in NMD. These patients may suffer from impaired coughing causing mucoid bronchus obliteration so that pressure-targeted systems may not guarantee minimum minute ventilation (76). While patients with neuro-muscular or thoraco-skeletal diseases can often be treated sufficiently with low tidal volumes, high pressure support may be needed in patients with COPD. In both groups, the treatment target is normalization of carbon dioxide so that the term "high pressure ventilation" is misleading. Inspiratory pressure levels above 20 mbar are not primarily intended, but may be required to overcome hypercapnia. Dreher *et al.* compared this approach of high-intensity pressure (NPPV: mean inspiratory pressure 28.6±1.9 mbar) with low-intensity pressure (NPPV: mean inspiratory pressures of 14.6±0.8 mbar) in patients with severe stable hypercapnic COPD. The high-intensity regime was associated with better compliance (mean difference of 3.6 h/d) and was superior in terms of controlling nocturnal hypoventilation (77). Murphy *et al.* demonstrated that the pressure component is the most important factor in controlling hypoventilation, while changes in backup frequency (high versus low) did not relevantly impact $PaCO_2$ (78).

Continuous positive airway pressure (CPAP) may be still the first therapeutical approach to patients with OHS. Due to the stabilization of upper airway obstruction, improvement of ventilation perfusion mismatch and lung mechanics, it might sufficiently normalize ventilation and oxygenation in a subgroup of OHS patients. Piper *et al.* randomized OHS patients to receive CPAP or bilevel ventilator support. They excluded patients with persisting severe nocturnal hypoxemia or sleep hypoventilation. Daytime carbon dioxide levels decreased similarly in both groups (79). However a substantial group of OHS patients did not sufficiently respond to CPAP, so that pressure support ventilation is the treatment of choice for the huge majority of patients with chronic hypoventilation. The heterogeneous responses to CPAP and pressure support ventilation might reflect the various contributions of the pathophysiological components (9). Similar to COPD, pressure support ventilation with volume assurance have not proven to generally be superior in OHS patients. Murphy *et al.* failed to demonstrate differences between automated volume assured pressure ventilation (AVAPS) and fixed-level pressure

support in super obese patients (BMI 50 ± 7 kg/m²) (72). However, hybrid modes and automatic algorithms may facilitate initiation and allow for individualized treatment (15).

NIV has proven to improve quality of sleep, nocturnal oxygen saturation, diurnal and nocturnal $PaCO_2$ and quality of life in a broad spectrum of various chronic hypoventilation disorders (67). Moreover, NIV may improve patients' survival (80). Non-invasive positive pressure ventilation added to standard treatment has proven to significantly improve survival in patients with COPD with $PaCO_2$ ≥7 kPa (51.9 mmHg) (80). Bourke *et al.* demonstrated improvement of the survival by approximately 7 months in ALS with orthopnea or daytime hypercapnia (81). The medium age of death in Duchenne's muscle dystrophy has ameliorated from 18-20 years to nearly 30 years under establishment of NIV (82). In addition, Nowbar *et al.* showed an increased mortality in patients with OHS. Twenty-three percent of OHS patients died within 18 months following hospital discharge as compared to 9% of patients with normocapnic obesity. Only 13% of the OHS patients were treated with NPPV. In addition, untreated OHS patients are more likely to require invasive ventilation and have prolonged hospital stays (83).

Most recently, a multicenter randomized controlled trial on the efficacy of NIV in severe stable COPD was performed. The investigators aimed at reducing hypercapnia by 20% or below a level of 48.1 mmHg. NIV was compared to standard treatment without ventilation. NIV significantly reduced 1 year mortality from 33% to 12% as compared to the control group. Therefore, from our point of view, NIV should be recommended to all COPD patients with chronic hypoventilation ($PaCO_2$ ≥50 mmHg) (80,84).

Conclusions

The huge variety of underlying diseases and pathophysiological factors urge the clinician to individualize treatment. NIV has become the therapy of choice of chronic hypoventilation but has to be adapted according to the specific needs of the patients.

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Footnote

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Clinical manifestations of sleep apnea

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Abstract: Obstructive sleep apnea (OSA) may manifest in a number of ways from subtle intrusion into daily life to profound sleepiness, snoring, witnessed apneas and other classic symptoms. Although there is increasing evidence suggesting OSA can adversely affect health in a variety of ways, this disorder remains underdiagnosed. The most well-escribed health consequences of OSA relate to the cardiovascular system. Hypertension and arrhythmias have a strong association with OSA, and evidence suggests that treatment of OSA in patients with refractory hypertension and in patients planning cardioversion for atrial fibrillation may be of particularly importance. Significant associations between heart failure and OSA as well as complex sleep apnea have also been well-described. Cerebrovascular insult, impaired neurocognition, and poorly controlled mood disorder are also associated with in OSA. Therapy for OSA may ameliorate atherosclerotic progression and improve outcomes post-cerebrovascular accident (CVA). OSA should be considered in patients complaining of poor concentration at work, actual or near-miss motor vehicle accidents, and patients with severe sleepiness as a component of their co-morbid mood disorders. The metabolic impact of OSA has also been studied, particularly in relation to glucose homeostasis. Also of interest is the potential impact OSA has on lipid metabolism. The adverse effect untreated OSA has on glucose tolerance and lipid levels has led to the suggestion that OSA is yet another constituent of the metabolic syndrome. Some of these metabolic derangements may be related to the adverse effects untreated OSA has on hepatic health. The cardiovascular, neurocognitive, and metabolic manifestations of OSA can have a significant impact on patient health and quality of life. In many instances, evidence exists that therapy not only improves outcomes in general, but also modifies the severity of co-morbid disease. To mitigate the long-term sequela of this disease, providers should be aware of the subtle manifestations of OSA and order appropriate testing as necessary.

Keywords: Obstructive sleep apnea (OSA); cardiovascular consequences of sleep apnea; metabolic derangements in sleep apnea; neurocognitive impact of sleep apnea

Introduction

Obstructive sleep apnea (OSA) manifests in a variety of ways from subtle intrusion into daily life that may be unrecognized by the patient and providers to profound sleepiness, snoring, witnessed apneas and other classic, more recognizable symptoms. Unfortunately the former presentation is the clinical norm. Symptom severity often

Table 1 Classic symptoms and findings in OSAS
Symptoms of OSAS
Snoring
Excessive daytime sleepiness
Choking or gasping at night
Night sweats
Neurocognitive impairment
Heartburn
Morning headaches
Maintenance insomnia
Erectile dysfunction
Nocturia
Exam findings of OSAS
Obesity
Enlarged neck circumference
Crowded upper airway
Hypertension
Accentuated P2 heart sounds (pulmonary HTN)
Retrognathia/overjet
Nasal obstruction
Decreased oxygen saturation
S3 heart sound (CHF)
Lower extremity edema (heart failure)
OSAS, obstructive sleep apnea syndrome; CHF, congestive heart failure.

progresses over years, leading to delayed diagnosis and allowing time for the disease to adversely affect health. Even with increasing awareness of the serious adverse consequences of untreated sleep apnea on patient outcomes and healthcare utilization, epidemiologic studies suggest that OSA is under-diagnosed (1,2). As the obesity epidemic continues to increase, the prevalence of OSA will likely continue to rise. Clinicians should be familiar with both the subtle and overt clinical manifestations of OSA to accurately identify patients at risk for the disease, order appropriate testing, and tailor therapy to the individual patient.

Clinical manifestations

A substantial amount of evidence supports an association between OSA and a number of disorders (3). Healthcare providers should strongly consider the possibility of OSA in patients with these co-morbidities particularly when found in conjunction with characteristic symptoms and physical

exam findings of OSA (*Table 1*). The evaluation of co-morbidities, symptoms, and anatomy is not only important when screening patients for OSA, but is also important with regard to determination of diagnostic testing and treatment modalities.

Cardiovascular associations

Adverse cardiovascular outcomes are perhaps the most well-described sequela of OSA. Although a number of cardiovascular risk factors (male sex, age, obesity, hypertension, and glucose intolerance) are found frequently in the OSA population, elevated risk is independently associated with OSA. The pathological relationship between sleep apnea and cardiovascular disease has been well-described (4) (*Figure 1*).

The relationship between OSA and hypertension was first suggested over 30 years ago (5). Subsequently numerous studies have attempted to determine the relationship between OSA and cardiovascular health. Co-morbid hypertension is a common finding in the sleep apnea population (6). The Wisconsin sleep cohort identified a dose-response relationship between sleep apnea severity and incident hypertension at 4 years follow-up independent of known co-founding risk factors (7). Another prospective cohort study reported this dose relationship between OSA and hypertension as well as the impact of continuous positive airway pressure (CPAP) therapy on the development of hypertension (8). This cohort of 1,889 patients without hypertension referred to a sleep center in Spain were divided into three groups: mild, moderate, and severe OSA. They were further classified as being treated with CPAP therapy, non-adherent to CPAP therapy, those who refused CPAP therapy, and those who were ineligible for CPAP therapy. Median follow-up period was 12 years. Incidence of hypertension increased with severity of sleep apnea. The authors found that the hazard ratios (HR) for incident hypertension were greater among patients with OSA ineligible for CPAP therapy [1.33; 95% confidence interval (CI), 1.01-1.75], among those who declined CPAP therapy (1.96; 95% CI, 1.44-2.66), and among those non-adherent to CPAP therapy (1.78; 95% CI, 1.23-2.58). The OSA group adherent to CPAP therapy had the lowest HR (0.71; 95% CI, 0.53-0.94) for the development of hypertension.

Not all studies have confirmed the association of hypertension and OSA. For instance, the Sleep Heart Health Study reported an increased incidence of hypertension with increasing baseline apnea hypopnea index (AHI); however, this finding lost statistical significance

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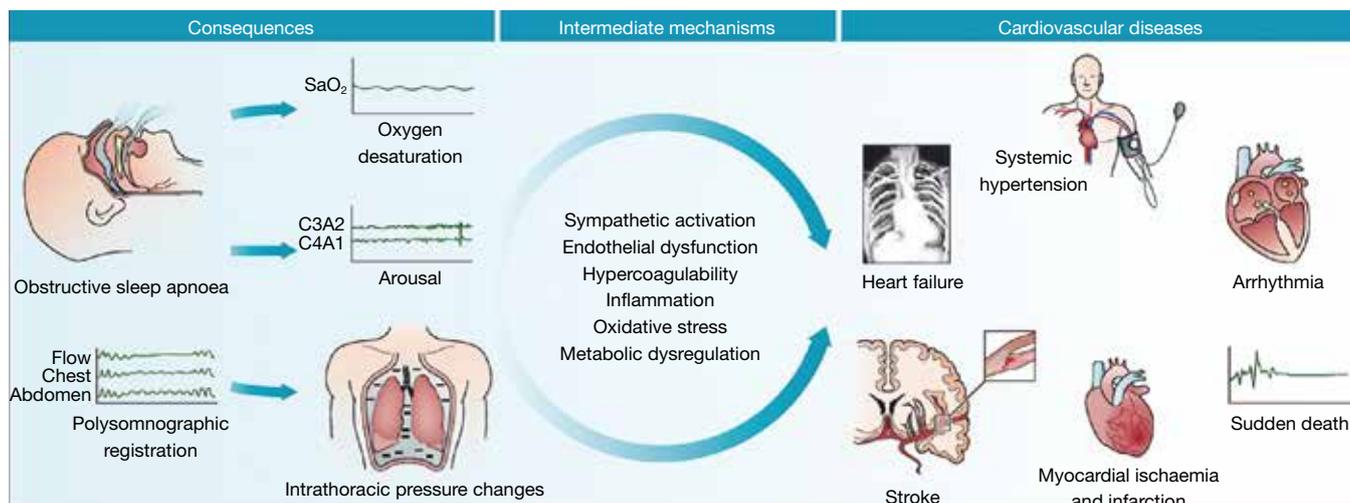
Figure 1 Pathological relationship between sleep apnea and cardiovascular disease [Reprinted with permission (4)].

after adjustment for baseline BMI (9). The weak association between OSA and systemic hypertension may be related to the older age of the cohort and the inclusion of a very large portion of participants with mild OSA.

A sub-group that deserves special consideration is the population with resistant hypertension, classically defined as individuals requiring three or more antihypertensive medications for blood pressure control. A case series reported by Gonçalves *et al.* compared 63 patients with resistant hypertension to a control group of 63 patients with controlled hypertension. The prevalence of OSA in patients with resistant hypertension was dramatic at 71% compared with an incidence of 38% in patients with controlled systemic hypertension (10). Recently published work from the Heart BEAT study suggests that severe OSA is associated with a much higher likelihood of persistent elevation in blood pressure in patients on multiple anti-hypertensive medications compared to moderate OSA (11). Based upon the above observations, screening for OSA in patients with resistant hypertension is reasonable; particularly in those with known risk factors for OSA (12,13).

The current evidence base supporting the role of OSA in the development of hypertension is somewhat controversial. Many questions remain unanswered. Treating moderate to severe sleep and symptomatic mild sleep apnea is indicated. Uncertainty arises in the asymptomatic population with mild sleep apnea and comorbid hypertension. The available data suggests that CPAP intervention leads to a modest reduction in blood pressure, and this reduction may be more prominent in the severe OSA population (14). Based on the

modest effect CPAP therapy would be expected to have in the mild OSA population, the impact of introducing this therapy in mild disease is likely minimal. Long-term follow-up data assessing the interaction of OSA, hypertension, and cardiovascular morbidity is lacking.

Altogether the evidence supports an association between OSA and the development of hypertension, particularly in more severe disease. It is likely that a range of individual susceptibility exists for the development of hypertension among patients at a given severity of OSA (15). The available data suggests improvement (albeit modest) in blood pressure with CPAP therapy; however, the introduction of CPAP therapy in a patient with mild sleep apnea, who is asymptomatic, is unlikely to have a clinically significant impact.

Arrhythmias, particularly atrial fibrillation, have been well-documented in the OSA population and are likely consequences of respiratory abnormalities and changes in autonomic tone during sleep. Contemporary case-control and cross-sectional studies as well as studies dating back 30 plus years support the relationship between OSA and cardiac arrhythmia (16,17). The rhythm disturbances documented in these studies include ventricular tachycardia or fibrillation, sinus arrest, atrioventricular conduction blocks, complex ventricular ectopy, supraventricular tachycardia, bradycardia, and atrial fibrillation (16-21).

Practitioners should be aware of the prevalence of OSA in patients with atrial fibrillation not only for diagnostic purposes, but also for potential treatment implications. Epidemiological evidence for this association is supported by

Mehra *et al.* analysis of the Sleep Heart Health Study data that reported the likelihood of atrial fibrillation is increased 4- to 5-fold in patients with sleep disordered breathing (SDB) (22). Other studies indicate that the risk of atrial fibrillation rises as the severity of sleep apnea increases (23,24). Evidence also suggests that treatment of sleep apnea decreases the risk of recurrence of atrial fibrillation following cardioversion or pulmonary vein isolation procedures (25-27). Kanagala and colleagues conducted a prospective observational study of 39 participants with OSA following cardioversion and the relationship between CPAP usage and atrial fibrillation/flutter recurrence (25). Twenty-seven of these patients were not on positive airway pressure therapy or had suboptimal adherence. Recurrence of AF at 12 months in these 27 patients was 82%, significantly higher than the 42% recurrence in the treated OSA group (n=12, P=0.013). Follow-up data were also obtained in 79 randomly selected post-cardioversion patients (controls) who did not have any previous sleep study; this group had a 53% recurrence of atrial fibrillation at 1 year (P=0.009). A recent study identified 62 patients referred for pulmonary vein isolation for treatment of atrial fibrillation who had sleep apnea confirmed by polysomnography (27). These participants were divided into a "CPAP user" group (n=32) and a "CPAP non-user" group (n=30). The CPAP user group had a lower rate of recurrent atrial fibrillation (28% *vs.* 63% for CPAP users *vs.* non-users; P=0.01). Also, a higher arrhythmia-free survival rate off antiarrhythmic drugs (66% *vs.* 33%; P=0.02) was reported.

Overall, the data suggests a strong relationship between atrial fibrillation and OSA. There is also some suggestion that patients undergoing attempts to re-establish normal sinus rhythm will have more success if their OSA is corrected with positive airway pressure therapy; however this is based on small observational trials and further study is needed.

SDB is frequently encountered in the heart failure population and may manifest as pure OSA or central apnea with Cheynne-Stokes respiration. This association was reported by Javaheri in 1998 when he evaluated 81 ambulatory men with congestive heart failure (CHF) (defined as left ventricular ejection fraction <45%) who agreed to undergo screening polysomnography (28). SDB (defined as an AHI ≥15 events per hour) was present in 51% of these individuals with a predominance of central sleep apnea in this cohort. A larger study undertaken by Sin and colleagues attempted to both better define the prevalence

of SDB in the heart failure population and identify risk factors associated with the development of central and/ or obstructive apnea in this population (29). A total of 450 participants were studied and, using a cut-off AHI of ten events per hour of sleep, 72% were identified as having SDB (prevalence was 61% and 53% for AHI cutoffs of 15 and 20 respectively). Of the 72% of participants identified with SDB, 148 had central sleep apnea; 168 had obstructive apnea; and 134 patients did not show evidence of SDB. Risk factors for the presence of central sleep apnea included male sex, hypocapnia during wakefulness, atrial fibrillation, and age >60 years old. Risk for OSA was increased in women >60 years old and was increased in men who had a BMI of >35 kg/m². SDB appears to be very common in the heart failure population with larger studies reporting a prevalence of at least 50%, even in patients who are optimally managed (30).

CHF and SDB seem to have a bi-directional relationship with one leading to and worsening the other. Kaneko *et al.* reported that CPAP therapy for OSA improves cardiovascular parameters including blood pressure and left ventricular systolic function in participants with CHF supporting the notion that obstructive apnea may represent a modifiable factor in the care of these types of patients (31). Support for the benefit of these physiologic improvements leading to clinically significant outcomes comes from Japan. Investigators followed 88 patients with moderate-to-severe sleep apnea with co-morbid heart failure who were randomized to CPAP therapy (n=65) and no therapy (n=23) (32). The CPAP therapy group was further subdivided into participants who were adherent and those who were non-adherent. The risk for death and hospitalization was increased in the untreated group (HR 2.03; 95% CI, 1.07-3.68; P=0.030) and in less compliant CPAP-treated patients (HR 4.02; 95% CI, 1.33-12.2; P=0.014). CPAP therapy appears to improve outcomes in patients with CHF, provided they are adherent to therapy.

Pulmonary hypertension (PH) is another cardiovascular disease with a well-established relationship to OSA. This association led to inclusion of OSA into group 3 (PH due to lung diseases and/or hypoxia) of the World Health Organization's PH classification system (33). PH in this population is typically only encountered in moderate-to-severe OSA and is generally mild. In one study, participants (n=220) referred for polysomnography were found to have moderate-to-severe OSAS (AHI >20) and agreed to right heart catheterization (34). Thirty-seven participants were found to have PH defined as a resting mean pulmonary

artery pressure (PAP) of at least 20 mmHg. Only 2 of these 37 participants had a mean PAP greater than 35 mmHg. Those with PH tended to have more obstructive lung disease, more severe sleep apnea, a higher arterial carbon dioxide tension, a lower arterial oxygen tension, and a higher BMI. Two subsequent studies evaluating participants with at least mild OSA and without COPD found approximately 20% had PH generally in the mild range (35,36).

Given the mild increase in pulmonary arterial pressure from OSA, one would expect that therapy in OSA patients with positive airway pressure would lead to only a modest reduction in PAP. Reports from 1970s and 1980s suggested improvement in PH with tracheostomy for OSA (37,38). The impact of contemporary management options for OSA such as positive airway pressure and oral appliance therapy in PH is not well studied. Arias and colleagues have published the only randomized control trial to date that has evaluated the impact of CPAP therapy on PH in OSA (39). They studied 23 participants in a crossover design with overall severe OSA (mean AHI 44 events per hour). Participants were randomized to sham CPAP or CPAP therapy and followed for 12 weeks. Baseline echocardiography was performed and then followed up after the treatment period. Ten out of 23 patients had PH at baseline. Two participants were excluded from the study because of CPAP non-adherence. Effective CPAP was associated with a significant although modest reduction in the values for pulmonary systolic pressure from 29 to 24 mmHg.

The recognition of adverse cardiovascular outcomes in patients with SDB is important not only due to the significant burden cardiovascular disease places on society, but also because effective treatment for OSA seems to curtail the cardiovascular risk in these patients. As described above, OSA is associated with many cardiovascular co-morbidities including hypertension, arrhythmias, CHF, and pulmonary HTN. The development of coronary artery disease may also be influenced by the presence of OSA (40). To explore the benefit of therapy, Marin *et al.* followed 277 age-matched and BMI-matched healthy men, 389 men with simple snorers, 409 patients with mild-moderate OSA, and 667 patients with severe OSA. The OSA group was further sub-divided into patients who used PAP therapy and those who did not use PAP (41). Their results revealed that the incidence of fatal and non-fatal cardiovascular events in untreated patients with severe OSAS was significantly higher than in healthy participants recruited from the general population matched for age and BMI. Importantly, treatment with CPAP significantly reduced cardiovascular risk in patients with severe OSAS.

Neurocognitive associations

Perhaps that best described neurologic sequela that also overlaps with cardiovascular consequences of OSA is stroke. Much work has been undertaken to understand the risk OSA portends for stroke. Other researchers have endeavored to study patients with SDB as a consequence of stroke.

The previously mentioned cardiovascular consequences of OSA, including atrial fibrillation, heart failure, and hypertension, all can lead to stroke. However, OSA in itself seems to lead to an increased risk for stroke independent of these other factors. Data from an observational cohort study of 1,022 patients referred for polysomnography found that OSA lead to a statistically significant increase in the risk of stroke or death (HR 1.97; 95% CI, 1.12-3.48; P=0.01) after adjustment for age, sex, race, smoking status, alcohol-consumption status, body-mass index, and the presence or absence of diabetes mellitus, hyperlipidemia, atrial fibrillation, and hypertension (42). Cross-sectional data from the Sleep Heart Health Study suggests patients with a higher AHI are at an increased risk for stroke. A subsequent report by Shahar *et al.* created four equal size groups (quartiles) for each index of sleep-disordered breathing using its percentile distribution in the sample (40). Participants in the highest quartile were found to have a 1.58 times greater odds for stroke compared to patients in the lowest quartile. A more recent analysis of prospective data from the Sleep Heart Health Study suggests that severe OSA is an independent risk factor in stroke for men (43).

OSA appears not only to be a risk factor stroke, but also seems to be highly prevalent in the post-cerebrovascular accident (CVA) population. Findings from a 2010 meta-analysis of 29 articles involving over 2,300 patients with stroke (ischemic, hemorrhagic or TIA) are presented below (44). The incidence of SDB with an AHI >5 was 72% and with an AHI >20 was 38%. A CNS insult is a known risk factor for central apnea, yet only 7% of the post-stroke patients with SDB had primarily central apnea in this analysis.

Treatment of SDB in patients whom have suffered a stroke may improve clinical outcomes. A prospective observational study involving 166 participants with stroke who subsequently underwent a sleep study reported that treatment of OSA improved mortality (45). Of these 166 individuals, 96 who had an AHI of 20 or greater were offered CPAP therapy. Participants with an AHI of 20 or greater and intolerant of CPAP (n=68) had an increased risk of mortality (HR 2.69; 95% CI, 1.32-5.61) compared with participants with an AHI of less than 20 (n=70). Individuals intolerant of CPAP therapy were also found to have an increased risk of mortality (HR 1.58; 95% CI, 1.01-2.49; P=0.04) when compared with participants with moderate to severe OSA who tolerated CPAP (n=28). No differences in mortality were identified among participants without OSA, those with mild disease, and those who were adherent to CPAP therapy. Immediate intervention for OSA in patients with stroke may impact short-term outcomes such as stroke severity and functional outcomes. Bravata *et al.* reported a population of 55 participants randomized to receive auto-CPAP therapy (n=31) within 2 days of stroke and a control group who received usual care (n=24) (46). Only those with polysomnorgraphic evidence of sleep apnea were continued on a full 30 days of auto-CPAP therapy. Individuals randomized to auto-CPAP had greater improvements in NIH Stroke Scale at 30 days (–3.0) compared to the control group (–1.0); P=0.03. Greater improvement was observed with increasing auto-CPAP use. Another small study evaluated introducing PAP therapy in patients with stroke undergoing inpatient rehabilitation (47). Participants were screened with PSG and 48 were identified to have significant sleep apnea (AHI ≥15). A total of 22 participants completed the study with CPAP and another 22 completed the standard care arm. CPAP was found to be of substantial benefit in terms of stroke recovery, functional and motor outcomes, and depression severity. CPAP therapy appeared to have minimal impact on cognitive outcomes.

The evidence that introduction of CPAP therapy to treat OSA improves stroke-specific outcomes remains small and mixed. Although the above studies suggest benefit of early CPAP intervention for patients with stroke and OSA, other small and similarly-designed studies failed to show significant impact of CPAP therapy on outcomes (48,49). To date, long-term data is lacking. Large randomized trials are needed to further assess the impact of treating OSA in patients with stroke.

OSA is associated with variable degrees of neurocognitive impairment. Traffic accidents and work performance deficits are surrogate markers of these neurocognitive impairments in the OSA population that may be reported in clinic. Antonelli *et al.* found that 1 in 4 patients with newly diagnosed OSAS had severe neurocognitive impairment, particularly with regard to inductive and deductive thinking as well as constructive ability (50). Beebe and colleagues performed a meta-analysis of 1,092 patients with OSAS. Their results suggested a substantial impact of OSA upon vigilance and executive functioning (51). A number of small studies have evaluated various domains of neurocognition and pooled data suggest OSAS has negative effects on inductive and deductive reasoning, attention, vigilance, learning, and memory (52,53). Not all patients with OSA develop neurocognitive impairment and risk factors for development have been described (52). As with the other described clinical manifestations of OSA, treatment of SDB may ameliorate the neurocognitive consequences of this disease (53).

There also seems to be a bi-directional between sleep health and mood disorders. For instance, a prospective cohort study by Peppard included 1,408 patients and found a 1.8 fold risk for the development of depression in patients with OSA (54). Conversely those with baseline depression had a 1.6 fold risk for the development of OSA. This longitudinal study also found a dose-response association between OSA and depression, suggesting a possible a causal link between these conditions. Other studies have not found a relationship between depression and OSA with a link between the two seeming particularly weak in men (55).

Despite the lack of definitive data confirming a relationship between OSA and depression, one disease may impact the other. A case series (n=17) explored the relationship between OSA and persistent major depression despite aggressive pharmacotherapy (56). Beck Depression Inventory (BDI) and Hamilton Rating Scale for Depression (HRSD) scores decreased significantly from 19.7 to 10.8 and 16.7 to 8.0 after 2 months of CPAP treatment (both P<0.01). These participants had severe OSAS at baseline with a mean pre-treatment AHI of 52. Further, there was a significant reduction in residual depressive symptoms, as measured by BDI and HRSD, and in subjective daytime sleepiness reflected in the Epworth score. These findings suggest improvement in residual depressive symptoms may correlate with the improvement of daytime sleepiness by correcting the underlying apnea in these patients. Another trial found not only that treating OSA with CPAP improved depression symptoms, but also the magnitude

of improvement of depression symptoms correlated with the degree of improvement CPAP had on OSA (57). Depression also appears to modify the clinical course of OSA. In a cross-sectional study of participants with untreated OSA (n=56), participants were asked to complete the Center for Epidemiologic Studies Depression (CESD), Profile of Mood States (POMS) and Medical Outcomes Studies (MOS) surveys (58). Hierarchical linear regression was applied to the data and OSA severity explained 13.4% (P=0.022) of variance while depression scores explained an additional 24.5% of fatigue. These results suggest that depressive symptoms are of greater importance than OSA severity indices in explaining fatigue in patients with these comorbid diseases.

OSA-induced cognitive insults (manifested by impaired judgment, slowed reaction time, impaired learning, and poor working memory) may compromise driving and performance at work (59). Patients with OSA often complain of difficulty staying on task at work, falling asleep inappropriately at their work space, memory trouble causing professional difficulties, and administrative disciplinary action related to poor performance. Neurocognitive insult from OSA is particularly worrisome in driving, specifically in professional drivers in whom lapses may have dire consequences. The estimated increased risk for a motor vehicle accident in OSA population is 2-7 times that of the unaffected population (60). Data also suggests that effective intervention with positive airway pressure therapy leads to a return to baseline driving capabilities (61); however, the overall risk for a motor vehicle accident in an individual patient is more difficult to assess (62). The official ATS clinical practice guideline suggests risk stratification of drivers, and states that "there is no compelling evidence to restrict driving privileges in patients with sleep apnea if there has not been a motor vehicle crash or an equivalent event" (62). The guideline defines a high-risk driver as a patient with OSA and significant daytime sleepiness, a recent motor vehicle crash or near miss accident due to sleepiness, fatigue, or in-attention (62). The best approach to driving status in the non-sleepy patient with OSA is yet to be determined.

Cerebrovascular insult and impaired neurocognition are commonly associated with OSA. Therapy for OSA may ameliorate atherosclerotic progression and improve outcomes post-CVA. OSA should be considered in patients complaining of poor concentration at work, actual or near-miss motor vehicle accidents, and patients with severe sleepiness as a component of their co-morbid mood disorder.

Metabolic associations

Evidence to date suggests that OSA has an adverse effect on individuals' metabolic profile, particularly in moderate-to-severe disease (63). The adverse impact of the OSA on glucose homeostasis, lipid metabolism, and fatty liver disease suggests that OSA should be considered as a potential component of metabolic syndrome (64,65).

Glucose metabolism is impacted by the presence of OSA. Alterations in the hypothalamic-pituitary-adrenal axis, aberrant sympathetic activation, induction of certain adipokines, and increased inflammation/oxidative stress caused by SDB have been identified as the intermediary steps between OSA and altered glucose metabolism (66). Investigators at Johns Hopkins used the frequently sampled intravenous glucose tolerance test in 118 non-diabetic participants with and without SDB to model the *in vivo* kinetics of glucose and insulin (67). They found a significant reduction in insulin sensitivity in patients with OSA, and this reduction in insulin sensitivity correlated with severity of SDB. These findings were independent of age, sex, race, and percent body fat.

Because physiologic abnormalities inherent in OSA are known to alter glucose metabolism, debate remains over the relationship between OSA and diabetes. . In a cross-sectional study of the Wisconsin Sleep Cohort, the odds ratio for having a physician diagnoses of diabetes mellitus with an AHI of 15 or greater versus an AHI of less than 5 was 2.30 (95% CI, 1.28-4.11; P=0.005) after adjustment for age, sex, and body habitus (68). However, these investigators did not find a statistically significant independent causal effect for the development of type II diabetes in their prospective analysis. Also, the incidence of diabetes over a 4-year follow-up period was not significantly related to the severity of SDB at the time of initial enrollment in the cohort when shared risk factors were taken into consideration. Taking the opposite approach to the Wisconsin Cohort, investigators analyzing data from the Sleep Heart Health study assessed the prevalence of OSA in patients with type 2 diabetes (69). Descriptive analyses revealed differences between diabetic and non-diabetic participants in respiratory disturbance index (RDI) (RDI ≥15 prevalence 24% in diabetic cohort versus 15.6% in non-diabetic cohort), as well as sleep stages, sleep time < 90% O_2 saturation, central apnea index, and periodic breathing (P<0.05, all). However, multivariable regression analyses that adjusted for age, sex, BMI, race, and neck circumference eliminated these differences for all sleep measures except percent time in rapid eye movement

(REM) sleep. Most studies to date have found an increased prevalence of diabetes in the OSA population; however, any elevated risk is typically nullified when adjusting for known cofounders. A recent cross-sectional analysis of 6,616 participants found an increased risk of diabetes in patients with OSA even after controlling for cofounders. Investigators applied multivariate regression analysis to assess type two diabetes mellitus prevalence according to OSA severity, as measured by the oxyhemoglobin desaturation index (70). Results from this evaluation found that the prevalence of type 2 diabetes increased with severity of OSA (6.6% in participants without OSA compared to 28.9% in those with severe OSA). Further this increased prevalence persisted in patients with mild, moderate, and severe OSA compared to the group without OSA after adjustment for cofounders [OR (95% CI) of 1.33 (1.04-1.72), 1.73 (1.33-2.25), and 1.87 (1.45-2.42) (P<0.001) respectively].Whether OSA has detrimental effects on diabetes risk remains unclear, but the high prevalence of diabetes in the OSA population is difficult to ignore.

Correction of apneic events with positive airway pressure seems to modestly improve glycemic control. One of the largest studies by Harsch *et al.* attempted to assess the impact of CPAP therapy on insulin sensitivity (71). Forty non-diabetic participants (AHI >20) were treated with CPAP. Prior to initiation of CPAP, 2 days after, and after 3 months of effective CPAP treatment, hyperinsulinemic euglycemic clamp studies were performed to evaluate insulin sensitivity. Increased insulin sensitivity occurred after 2 days and remained stable after 2 months. Improvement in insulin sensitivity was most pronounced in the non-obese (BMI <30 kg/m^2) participants suggesting that obesity plays a larger role in insulin resistance than apnea. Based on these results, however, non-obese patients may expect a more dramatic increase in insulin sensitivity following CPAP therapy. Two observational trials involving patients with moderate-to-severe OSA suggested modest improvements in HbA1C levels (~0.2% decrease in both studies) following 3-5 months of CPAP therapy (72,73). Improvement in insulin sensitivity and glycemic control with correction of apnea via positive airway pressure therapy has not been universally confirmed, but overall positive airway pressure does appear to have some impact on glycemic control; particularly in those with severe OSA (74,75).

OSA appears to have an impact on lipid metabolism with studies suggesting functional abnormalities in high-density lipoproteins (HDL) and elevations in total cholesterol, low-density lipoproteins (LDL), and triglyceride levels (65,76,77). Compared to the other clinical abnormalities associated with OSA, much less data exists regarding this association. The pathobiology is just beginning to be understood and current data implicates OSA in decreased lipoprotein clearance, increased lipolysis, and enhanced hepatic lipid output (78). Further support for an interaction between OSA and lipid metabolism comes from an observed relationship between severity of desaturation and triglyceride and LDL levels in a murine model (79). Nominal data also exists linking CPAP therapy with improvement in dyslipidemia. A study in Greece evaluated serum risk factors at baseline and 6 months in 53 participants newly diagnosed with OSA (80). They were non-smokers with no comorbidities or medication use. These serum cardiovascular risk factors included high-sensitivity C-reactive protein, homocysteine, total cholesterol, triglycerides, HDL, and LDL. These participants were classified into three groups: group 1 (n=20), good compliance (≥4 h use per night); group 2 (n=19), poor compliance (<4 h use per night); and group 3 (n=14), refusal of CPAP treatment. Group 1 had significant decreases in hs-CRP (P=0.03), homocysteine (P=0.005), total cholesterol (P=0.021), and total cholesterol/HDL-C ratio (P=0.018). Group 2 subjects showed a decrease in homocysteine levels (P=0.021) only. There were no significant changes from baseline in group 3.

Recent interpretation of the available data has led to the suggestion that OSA is yet another constituent of the metabolic syndrome. Adult Treatment Panel III defines the metabolic syndrome as the presence of any three of the following five traits: (I) abdominal obesity; (II) elevated serum triglycerides or drug treatment for elevated triglycerides; (III) low serum HDL cholesterol; (IV) hypertension or drug treatment for elevated blood pressure; and (V) elevation in fasting plasma glucose or drug treatment for elevated blood glucose (81). The International Diabetes Federation (IDF) updated their metabolic syndrome criteria in 2006 with central obesity as an essential element in their definition (82). The correlation between OSA and visceral fat deposition has been well described. Work by Vgontzas *et al.* analyzed the fat deposition of 14 obese participants with OSAS and ten obese participants without OSA (83). No differences between the two groups in terms of total body fat or subcutaneous fat were identified. The OSA group compared to obese controls had a significantly greater amount of visceral fat (P<0.05 at levels measured). Further, BMI and the amount of subcutaneous

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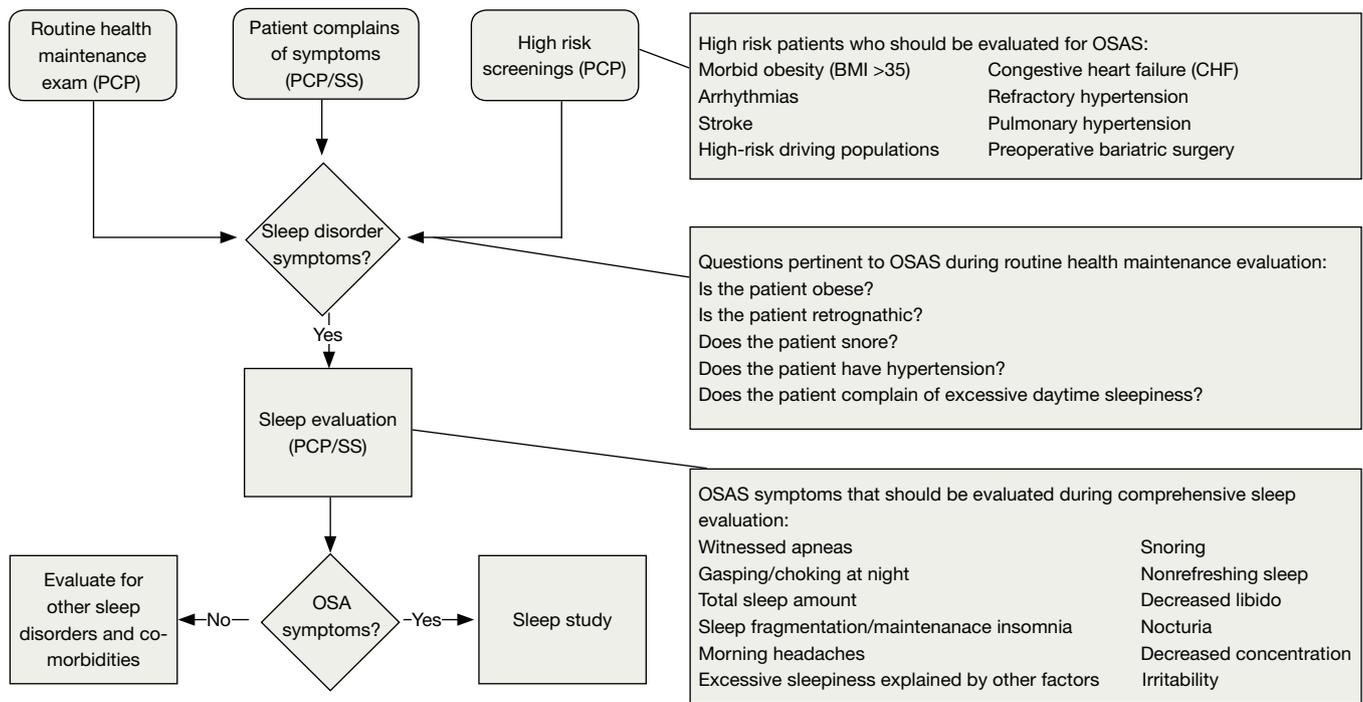
Figure 2 Flow chart for screening of patients suspected of having obstructive sleep apnea (OSA) (89). OSAS, obstructive sleep apnea syndrome.

fat did not correlate with metrics of OSA severity, whereas visceral obesity was significantly correlated with increasing AHI and degree of nocturnal hypoxemia. The fundamental role of central obesity in OSA and metabolic syndrome as well as the previously discussed associations between OSA and the other defining traits of the metabolic syndrome (hypertension, dyslipidemia, and hyperglycemia) suggests a possible common genetic determinant of this phenotype. There is currently inadequate data from humans, due to the inherent challenges of separating out effects of SDB and obesity, to completely understand the independent impact OSA has on metabolism.

Liver steatosis is a known manifestation of insulin resistance and metabolic syndrome. Recent investigations into the impact OSA has on individuals with the metabolic syndrome and the progression from asymptomatic hepatic steatosis, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and finally cirrhotic liver disease have been undertaken. Unfortunately a paucity of data currently exists, but the data pool is expanding. Human studies are limited by less invasive evaluations of the liver (imaging, liver enzymes) and do not distinguish between hepatic steatosis, NAFLD, and NASH. Most histopathologic data come from OSA patients with severe morbid obesity that undergoes a liver

biopsy during bariatric surgery (84). OSA may lead to hepatic steatosis. A study by Tanné et al. evaluated liver damage in 163 consecutive nondrinking patients who had undergone polysomnographic recording for clinical suspicion of OSA (85). Liver enzymes were obtained in all patients, and liver biopsy was offered to patients with elevated liver enzymes. Participants with severe OSA (defined as an AHI >50 events per hour) had a higher percentage of steatosis as well as liver necrosis and fibrosis, despite having a similar BMI. Other small studies have not demonstrated an association between OSAS and hepatic steatosis (86,87). Although a study of 40 obese Japanese men with sleep apnea reported that an intervention with nasal CPAP to correct SDB ameliorated liver injury, independent of changes in adiposity (88).

OSA screening

As outlined above OSA can have significant health consequences. Appropriate screening for OSA by primary care providers or sleep specialist is essential to establishing a diagnosis and initiating appropriate treatment. Health care providers screening patients in clinic should take into consideration the classic signs and symptoms as well as high risk populations as outlined in *Figure 2*.

Conclusions

OSA can present in a variety of ways. OSA can significantly impact co-morbid disease leading to worse outcomes in this patient population. Providers should be vigilant of some the more subtle clinical manifestations of OSA. The cardiovascular, neurocognitive, and metabolic manifestations and can have a significant impact on patient health and quality of life Evidence exists that therapy not only improves outcomes in general, but modifies the severity of co-morbid disease as well. As the obesity epidemic increases, providers will be faced with more patients suffering from OSA. To mitigate the long-term sequela of this disease, providers should be prepared to order appropriate screening tests and provide proper longitudinal management.

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Footnote

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Diagnostic approaches to respiratory sleep disorders

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Abstract: Sleep disordered breathing (SDB) comprises a number of breathing disturbances occurring during sleep including snoring, the obstructive sleep apnoea/hypopnea syndrome (OSAHS), central sleep apnoea (CSA) and hypoventilation syndromes. This review focuses on sleep disordered breathing and diagnostic approaches in adults, in particular clinical assessment and overnight assessment during sleep. Although diagnostic approaches to respiratory sleep disorders are reasonably straightforward, they do require a degree of clinical acumen when it comes to assessing severity and management options. Diagnosing respiratory sleep disorders on clinical features alone has limitations. Monitoring and measuring respiration during sleep has undergone many advances in the last 40 years in respect of quality and validity, largely regarding OSAHS. Despite the improvement in our diagnostic standards and recognition of sleep disordered breathing, many limitations still need to be overcome. Apart from assessing the individual patient, population screening for sleep disorders continues to preoccupy health professionals and policy makers in many countries. Research in the field is pushing current boundaries in terms of simplifying diagnosis and enhancing screening for sleep disordered breathing in large populations. At present, a number of these newer approaches require further validation.

Keywords: Clinical assessment; diagnosis; objective assessment; population screening; respiratory sleep disorders; sleep apnoea; sleep disordered breathing (SDB)

Introduction

Sleep disordered breathing (SDB) comprises a number of sleep related breathing disturbances: snoring, the obstructive sleep apnoea/hypopnea syndrome (OSAHS), central sleep apnoea (CSA), hypoventilation syndromes and other rarer forms of disturbed breathing (1).

The commonest form of sleep disordered breathing within industrialised communities is the OSAHS affecting at least 2-4% of the middle aged population (2). The definition of OSAHS is made on the basis of symptoms of daytime sleepiness and objective measures of disordered breathing during sleep. Recurrent upper airway (UA) obstruction during sleep, resulting in repetitive apnoeas accompanied by oxygen desaturation and arousal from sleep is the chief diagnostic characteristic of OSAHS (2).

OSAHS leads to widespread physiological changes which may contribute to the development of both cardiovascular and cerebrovascular morbidity (2), in addition to diurnal sleepiness and cognitive impairment. Currently, the standard treatment for moderate to severe OSAHS is in the form of splinting the airways mechanically using compressed air delivered through a nasal or full face mask worn during sleep [continuous positive airway pressure (CPAP)] (2).

Table 1 lists the current classification of breathing disturbances during sleep according to the International Classification of Sleep Disorders 3rd edition (ICSD-3),

Table 1 Classification of sleep related breathing disorders according to the ICSD-3 classification of sleep disorders manual (1)

Disorder	ICD-10-CM code
Obstructive sleep apnea disorders	
Obstructive sleep apnea, adult	G47.33
Obstructive sleep apnea, pediatric	G47.33
Central sleep apnea syndromes	
Central sleep apnea with Cheyne-Stokes breathing	RO6.3
Central apnea due to a medical disorder without Cheyne-Stokes breathing	G47.37
Central sleep apnea due to high altitude periodic breathing	G47.32
Central sleep apnea due to a medication or substance	G47.39
Primary central sleep apnea	G47.31
Primary central sleep apnea of infancy	P28.3
Primary central sleep apnea of prematurity	P28.4
Treatment-emergent central sleep apnea	G47.39
Sleep related hypoventilation disorders	
Obesity hypoventilation syndrome	E66.2
Congenital central alveolar hypoventilation syndrome	G47.35
Late-Onset central hypoventilation with hypothalamic Dysfunction	G47.36
Idiopathic central alveolar hypoventilation	G47.34
Sleep related hypoventilation due to a medication of Substance	G47.36
Sleep related hypoventilation due to a medical disorder	G47.36
Sleep related hypoxaemia disorder	
Sleep related hypoxaemia	G47.36
Isolated symptoms and normal variants	
Snoring	R06.83
Catathrenia	
ICSD, International Classification of Sleep Disorders	

published in 2014 (1). The clinical presentation and diagnostic criteria of abnormal breathing in sleep are different for adult and paediatric cases. In this review the focus will be on sleep disordered breathing in adults, in particular, OSAHS, CSA and sleep related hypoventilation syndromes.

Clinical assessment of sleep related breathing disorders

The obstructive sleep apnoea/hypopnoea syndrome

The pathophysiology of OSAHS is based in UA occlusion during sleep (3). Occlusion of the UA is termed an apnoea if complete and hypopnoea if partial (see *Figure 1*). An obstructive apnoea is defined as a cessation of airflow with continued effort for at least 10 seconds. Apnoeas/hypopnoeas are often, associated with an electroencephalographic (EEG) arousal at their termination and with a drop in oxygen saturation (4-7). The standardisation of hypopnoea definitions has fluctuated over time. However, the definition adopted by the American Academy of Sleep Medicine (AASM) in 2007 is a 30-50% reduction in thoraco-abdominal movement for at least 10 seconds from the preceding stable baseline with either an accompanying 3% or 4% desaturation or an arousal (5). In reality, a number of definitions are likely to be in current use, some idiosyncratic to a given sleep laboratory and potentially encompassing older definitions of hypopnoeas, including the 'Chicago Criteria' and a definition provided by the 2001 AASM position paper (8). Thus, the effect on the overall apnoea/hypopnoea index (AHI) which is the metric employed to define OSAHS severity, is highly variable and several studies have demonstrated that diagnosis can differ as a result (9,10). The American Academy of Sleep Medicine Task Force in 1999 (4) defined OSAHS severity on the basis of two components: severity of daytime sleepiness and the AHI. Sleepiness and breathing events are rated separately. Sleep-related obstructive breathing events are rated as mild (5-15 events per hour of sleep), moderate (15-30 events per hour of sleep) or severe (greater than 30 events per hour of sleep) (4). This largely constitutes a good general working definition of the disorder but does not account for age- or gender-related changes in sleepiness and sleep-disordered breathing. It is important to note, that there are very few normative data for either the general population, let alone more specific populations e.g., the elderly, people with intellectual disability (3).

Presenting symptoms of OSAHS can be divided into those that manifest themselves during sleep and those which are present during wakefulness (*Table 2*). The most common complaint is excessive day time sleepiness (EDS). A multi-centre cohort study published in 2008 suggested that apnoea and sleep disruption were not the primary determinants of EDS, although patients with EDS had longer sleep duration, increased slow wave sleep and sleep
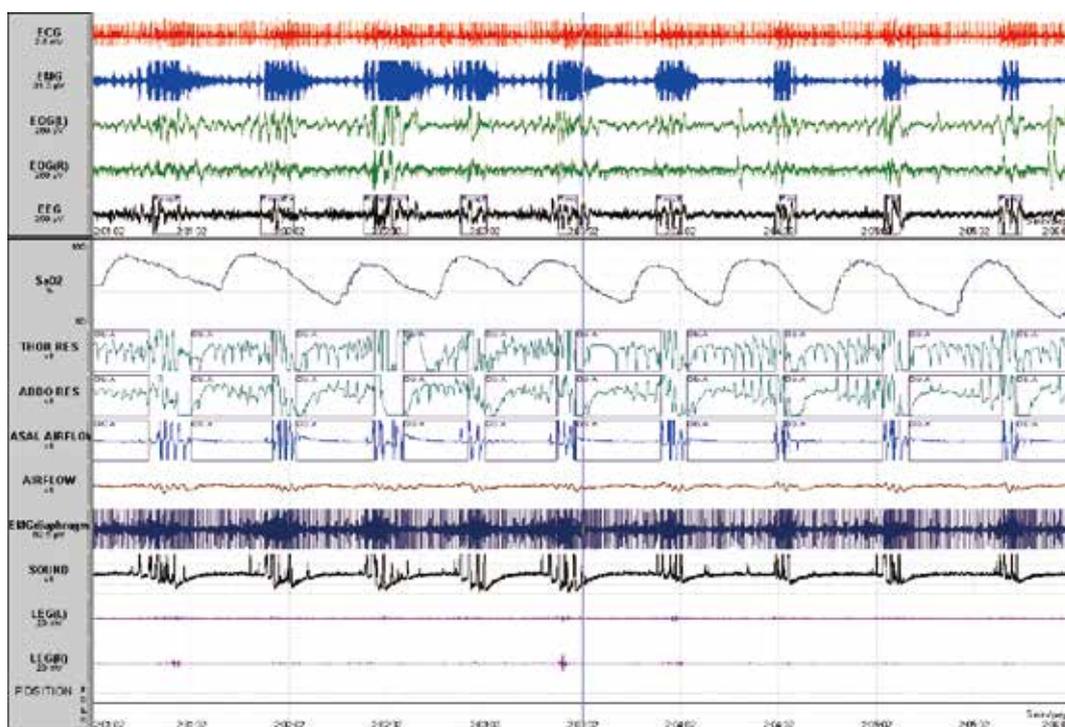
Figure 1 Five-minute page from a polysomnogram showing obstructive apnoeas (Ob.A) recorded on thoracic, abdominal bands and airflow associated with desaturation and arousals at the termination of the events on electroencephalogram. ECG, electrocardiogram; EMG, electromyogram; EOG, electro-oculogram; EEG, electroencephalogram; SaO₂, pulse oximetry saturation; Thor Res, thoracic band; Abdo res, abdominal band.

Table 2 Symptoms of the obstructive sleep apnoea/hypopnoea syndrome	
During sleep	**Awake**
Non-restorative sleep	Daytime sleepiness
Witnessed apnoeas by bed partner	Lack of concentration
Awakening with choking	Cognitive deficits
Nocturnal restlessness	Changes in mood
Vivid dreams	Morning headaches
Gastroesophageal reflux	Dry mouth
Insomnia with frequent awakenings	Impotence or decreased libido
Nocturia	
Hypersalivation	
Diaphoresis	

fragmentation (11).

EDS can be a symptom of many different pathologies. Important differential diagnoses for EDS include: sleep deprivation, metabolic disorders and depression (*Table 3*).

Nocturnal symptoms of OSAHS are sometimes apparent to the patient but generally are reported by the bed partner. Most commonly reported are snoring, snorting, choking attacks terminating a snore and apnoeas. An absence of snoring does not exclude a diagnosis of OSAHS, but virtually all patients snore. Apnoeic episodes are reported by approximately 75% of bed partners. Many patients report waking up with choking or gasping but remain unaware of their disordered breathing. Those who are aware of these events sometimes choose to delay sleep onset and can present with 'paradoxical insomnia'. A number of clinical features are associated with OSAHS; many occur in combination and may also be subtle (*Table 4*). The predictive value of any single feature is limited (12).

When considering the clinical presentation of OSAHS, it should be borne in mind that the bulk of the scientific literature has to date focussed on middle-aged men who have for the most part been overweight. The female phenotype is less clearly defined and male bed partners are less likely to report snoring and apnoeas to a health professional. Female OSAHS patients may present with less

Table 3 Differential diagnosis of excessive daytime sleepiness

Obstructive sleep apnea/hypopnea syndrome (OSAHS)
Upper airway resistance syndrome (UARS)
Narcolepsy—central nervous system hypersomnolence
Periodic leg movements during sleep
Insufficient sleep syndrome
Alcohol, medication and drug use
Schedule disorders/shift work
Chronic pain and discomfort
Delayed sleep phase syndrome
Severe lung disease
Neuromuscular disease
Metabolic abnormalities
Vitamin and trace element deficiencies (if profound)
Depression

Table 4 Commonly encountered clinical features of obstructive sleep apnoea/hypopnoea syndrome

Obesity (particularly central, BMI >30 kg/m^2)
Large neck circumference (>40 cm)
Narrow mandible, narrow maxilla
Retrognathia
Dental malocclusion, overbite
Reduced nasal patency
High and narrow hard palate
Elongated and low-lying uvula
Enlarged tonsils and adenoids
Macroglossia

typical and more diffuse signs such as headache, insomnia and mood disturbances (13). Age at presentation will also determine clinical symptoms. Younger patients may have behavioural and cognitive disturbances, rather than EDS as a primary presenting feature. In the elderly, co-morbidities and lifestyle changes (e.g., diurnal napping) may cloud symptomatology (14). Patients with intellectual disability may have presenting features more akin to children with OSAHS (15).

Clinical examination of a patient presenting with a history of obstructive respiratory events during sleep should include cardio-respiratory auscultation, blood pressure measurement, examination of the oral cavity, noting the presence of teeth and dentures. It is also highly

recommended to comment on tonsil size, tongue size and architecture of the hard palate and faucial pillars. A simple way of recording this information is by using the Mallampati score, first devised for assessing ease of intubation of patients for purposes of anaesthesia (16). There have been several studies now which have shown an independent association between Mallampati score and presence or severity of OSAHS (17). Another score which is also useful is the Friedman score, used more frequently within Ear Nose and Throat practice (18).

Since OSAHS is an independent risk factor for hypertension and may contribute to and co-exist with a number of co-morbidities, it is now increasingly being recommended, that these should be examined for and addressed as well. A suggested approach would include screening for at least the following: hypertension, cardiac hypertrophy and atrial fibrillation, evidence of atherosclerosis (coronary or peripheral), COPD, depression, obesity (waist/hip ratio; plasma lipids, hepatic enzymes, liver ultrasound), Type II diabetes mellitus, insulin resistance and metabolic syndrome.

History and clinical examination alone, including blood pressure and body mass index (BMI) measurement can predict the presence of OSAHS in about 50% of patients attending a specialised sleep clinic (12). Further, definitive diagnosis requires additional monitoring during sleep.

Central sleep apnoea (CSA)

Overall, 10% of breathing disturbances during sleep are secondary to CSA (1). CSA is commoner in the elderly population and in males (19). The reason for this observation may lie in the fact that women have a lower apnoeic threshold which stabilises respiration (20).

CSA is defined by recurrent cessation of airflow with simultaneous cessation of respiratory effort (5,6,7). The primary pathophysiology is related to a reduction in or complete lack of ventilator drive/impulse generation. *Table 5* lists the commonest causes of CSA, including hypoventilation syndromes (discussed below).

The term CSA includes a number of different disorders characterised by either hypo- or hyperventilation and sensitivity to carbon dioxide (CO_2). These are primary CSA, Cheyne-Stokes respiration (CSR), CSA secondary to high-altitude (periodic breathing), CSA secondary to brain-stem lesions and also secondary to drugs, most commonly opioids (1).

Clinical presentation of patients with CSA will also vary in respect of the underlying disorder, including heart

Table 5 Physiological classification of CSA based on wakefulness CO_2 levels (some overlap) [Based on reference (21)]

Hypercapnic CSA
"Won't breathe"
Brainstem lesions
Congenital central hypoventilation
Opioids
Obesity hypoventilation
"Can't breathe"
Neuromuscular disorders
Chest wall syndromes
Nonhypercapnic/Eucapnic
Cheyne-Stokes breathing
CSA, central sleep apnoea.

failure, atrial fibrillation (22) and stroke (23), chronic opioid use (24), family history, neurological disorder, or immediate environmental situation e.g., living at high altitude.

Generally, the symptoms of CSA are often subtle and nonspecific and include frequent awakenings during sleep, paroxysmal nocturnal dyspnoea in association with heart failure, fatigue and EDS. The latter symptom is often less marked than in obstructive sleep apnoea. Snoring is not a feature of this condition unless UAs obstruction is also present (1).

In clinical practice, the most common form of CSA occurs secondary to poorly controlled heart failure (25). In this disorder CSR is the primary diagnostic sign (see *Figure 2*). CSR is characterised by recurrent apnoeas and hypopnoeas occurring in a crescendo-decrescendo pattern of flow and effort. In CSR, cycle-length averages about 60-90 seconds, compared to other forms of CSA when it is shorter (26).

Figure 2 Cheyne-Stokes respiration on a 10-minute page from a polysomnogram during N2 sleep. EMG, electromyogram; ECG, electrocardiogram; Thor, thoracic band; Abdo, abdominal band.

In CSA, arterial blood gas examination is a useful investigative tool. Results can assist with a physiological classification based on wakefulness levels of CO_2, although there is some overlap between the different types of disorders. The hypercapnic CSA patients are those who "won't breathe" and include people with brain stem lesions, congenital central hypoventilation, opioids and obesity hypoventilation. Those who "can't breathe" are more likely to be those with neuromuscular disorders and chest wall syndromes. Non-hypercapnic or eucapnic forms of CSA include CSR and idiopathic CSA (see *Table 5*).

Hypoventilation syndromes

The primary pathophysiological trait of hypoventilation syndromes is a $PaCO_2$ >45 mmHg on arterial blood gas during sleep, which eventually manifests as abnormally elevated $PaCO_2$ during wakefulness (1). Arterial oxygen desaturations less than 90% for longer than 5 minutes during the sleep period with a nadir of less than 85% also characterise hypoventilation (27). Hypoventilation first becomes apparent in REM-sleep and as it progresses, will occur throughout all sleep stages.

Obesity hypoventilation syndrome (OHS)

OHS deserves special consideration as it is increasingly common with a prevalence of patients presenting to sleep clinics in industrialised countries ranging from 10% to 20% (1). Fifty percent of hospitalised patients with a BMI of greater 50 kg/m^2 are likely to have OHS. An overall estimate has suggested that 0.15-0.4% of the population has OHS with the incidence increasing as obesity increases in the general population. The diagnosis is important in that those with OHS have a 23% mortality at 18 months, which falls to 3% with appropriate treatment (1,28).

OHS is defined by a BMI greater than 30 kg/m^2, a $PaCO_2$ >45 mmHg and a FEV1/VC ratio >60% on respiratory function testing (1,28). The triad of obesity, hypersomnolence and awake hypercapnia in the absence of an alternative neuromuscular, mechanical or metabolic explanation for hypoventilation are the cornerstones of this diagnosis. Eighty to 90% of people with OHS also have UAs obstruction (1).

Generally, patients will report EDS. Other complaints include morning headache and loss of appetite in the morning which lifts throughout the day, mood disturbances and neurocognitive dysfunction (1,28).

Clinical examination is as above for OSAHS, but in severe cases, additional clinical signs should be noted including generalised plethora, scleral injection, peripheral oedema and signs of right heart failure and pulmonary hypertension (1).

Apart from arterial blood gases, other investigations should include assessment for polycythaemia, performing electrocardiogram (ECG) and echocardiography, looking for evidence of right ventricular dysfunction or failure, pulmonary hypertension and right ventricular hypertrophy.

Unfortunately, even severe OHS can go undetected for long periods of time until a catastrophic event such as myocardial infarction or a severe infective illness tips the balance into acute-on-chronic respiratory failure requiring urgent hospitalisation and high-level care (1,28).

Objective assessment of respiratory sleep disorders

Definitive diagnosis of most respiratory sleep disorders requires objective recording and measurement of sleep and breathing during sleep, in addition to a measure of daytime sleepiness (either objective or subjective) and symptoms of sleepiness.

Assessing day time sleepiness

The assessment of sleepiness is complex. Sleepiness *per se* is difficult to define objectively, but can be classified using a number of methods: behavioural measures (e.g., observation of yawning frequency, actigraphy, facial expression), performance tests (e.g., a driving simulator, psychomotor vigilant tests and reaction time tests), self-evaluation by rating scales [e.g., the Stanford Sleepiness Scale (SSS), Epworth Sleepiness Scale (ESS)] or direct electro-physiological measures (e.g., multiple sleep latency testing and multiple wakefulness testing, pupillometry and cerebral evoked potentials) (29).

In clinical practice, the most commonly used and best validated scale for assessing day time sleepiness is the ESS (30). An ESS score greater than 11 out of 24 (maximum score) is generally indicative of abnormal levels of daytime sleepiness, irrespective of age. The ESS can be applied in all disorders of sleepiness, including disorders of central hypersomnolence as well as circadian rhythm disorders.

The ESS aims to measure the general level of diurnal sleepiness as a stable individual characteristic and has satisfactory test/retest reliability. However, it can be misinterpreted and the questionnaire itself filled out inappropriately by patients, sometimes to minimise

Table 6 American College of Chest Physicians and the American Sleep Disorders Association classification of sleep monitoring devices [Based on reference (37)]

Type 1: full attended PSG (≥7 channels) in a laboratory setting
Type 2: full unattended PSG (≥7 channels)
Type 3: limited channel devices (4-7 channels)
Type 4: 1 or 2 channels usually using oximetry as one of the parameters
PSG, polysomnography.

symptomatology when a sleep disorder diagnosis might potentially threaten livelihood, e.g., a sleepy truck driver who then needs annual monitoring of adequate treatment of OSAHS to fulfil driving license regulations. A pictorial Epworth Sleepiness Score published recently has potentially made interpretation of the questions easier (31). However, the population in which this version of the ESS is applied may not necessarily make this a more workable tool.

Objective tests of daytime sleepiness include the multiple sleep latency test (MSLT) and the maintenance of wakefulness test (MWT) (32). The MSLT, originally designed to facilitate the diagnosis of narcolepsy, is also used in the assessment of disorders presenting with EDS. The central premise of the MSLT is that the sleepier a subject is, the more quickly he/she will fall asleep (32). In respect of routine use in respiratory sleep disorders, it is not recommended but can be useful if there are ongoing issues with EDS which have not been addressed through adequate treatment or when co-pathologies are suspected. The MWT measures the ability to stay awake for a defined period of time in a laboratory-controlled, stimulus-free environment (32). Again, this test is not useful in the routine management of respiratory sleep disorders, but can be utilised in the context of specific clinical questions e.g., has the patient responded sufficiently to treatment?

A number of additional computer-based assessments of visual attention and reaction times such as the OSLER test are also available (33). It is important to note, that despite their diagnostic usefulness under certain circumstances, no test of sleepiness can reliably predict sleepiness and performance in real-life situations with certainty.

Recording and measurement of sleep and breathing

Overnight polysomnography (PSG) is the most widely used method for the diagnosis of sleep related respiratory disorders. Currently it is considered the "gold standard" by which newer developments in the measurement of breathing during sleep have been assessed. Whether this is strictly appropriate is debatable. The PSG is designed to simultaneously monitor the following physiological recordings: nasal and/or oral airflow; thoracoabdominal movement; snoring; EEG; electro-oculogram (EOG); electromyogram (EMG); oxygen saturation and occasionally transcutaneous CO_2 (4,6,7).

The recording of any abnormal movements by additionally using video may help identify changes in airflow or desaturations. Signal collection and interpretation are usually computerised, but manual scoring of the trace should still be performed using guidelines for interpretation of the EEG (4,6,7). The scoring of respiratory and other events is likewise subject to guidelines. There have been a number of changes in guidelines over the last decade. Initially, the 1968 Rechtschaffen and Kales (34) rules for the scoring of EEG remained unchallenged until 2007 when the AASM revised the criteria and in some respects simplified EEG montage and scoring (6). There have been additional overviews of scoring since then (7). The standardisation of respiratory scoring using PSG has been the primary improvement, but variation still remains. Additionally, night to night variability in PSG makes it possible for a single study to underestimate the severity of OSAHS. A negative PSG should be viewed with scepticism if the clinical suspicion of OSAHS remains high (35).

Split-night studies in which the first half of the study night is used for diagnosis and the second for monitoring treatment response using CPAP are also used. Split-night studies are considered accurate and cost-effective when criteria for conducting them are met (36).

More recent introductions to the assessment of sleep disordered breathing have been cardio-respiratory monitoring devices or polygraphy (PG). These devices are also known as Type 3 devices using the AASM classification (*Table 6*) (37). PG comprises the measurement of airflow, respiratory effort, oxygen saturation and heart rate, to the exclusion of EEG. The great advantages of these systems are price, portability and convenience to the patient (38). Although automated scoring algorithms exist, manual scoring and overview should be undertaken. The results are expressed as A + H per time in bed as it is impossible to determine for sure without EEG how long the patient has been asleep. This can lead to both under- and overestimation of the actual severity of

sleep disordered breathing. There are no clear clinical guidelines for the scoring of respiratory events using PG. The AASM guidelines (4-7), have a definition for the scoring of obstructive apnoeas and hypopneas as well as central events. However, these are based on the presence of EEG. Additionally, accuracy of Type III equipment can differ, depending on the manufacturer. There are currently two possibilities for scoring respiratory events utilising Type III systems: (I) Ignore any events that do not have a desaturation; (II) score all events with or without desaturation 'in the hope' that there may be an associated arousal with events without desaturation.

Even more simplified systems have been utilised in screening and diagnosis of sleep disordered breathing, most commonly overnight oximetry. However, single channel oximetry should never be seen as a substitute for either in-lab PSG or PG. There are limitations to the technique, in particular, failure to detect apnoeas/hypopnoeas although the pattern may be suggestive (39,40). Furthermore, oxygen desaturation is not necessarily a marker of apnoeic episodes and may be the results of other disorders including hypoventilation without UAs obstruction. Oximetry can be used to monitor patients who have been appropriately diagnosed with a respiratory sleep disorder in respect of progression of disease or treatment response.

Other forms of single-channel devices comprise measurement of nasal flow to characterise apnoeas and hypopnoeas. A recent multi-centre study testing a home single-channel nasal pressure device in over 700 patients with sleep disordered breathing showed very good diagnostic accuracy and cost-effectiveness compared to PSG and PG, particularly when the traces were manually scored (41).

There have been newer additions to the diagnostic armamentarium and approach to the physical space where diagnosis is undertaken. These include not only in-lab studies or home-based monitoring, but also telemonitoring via remote control and remote monitoring of diagnostic studies. Currently, there are few well-conducted trials reported, but preliminary results suggest that this is a promising area which may further increase convenience to patients and decrease the costs to a diagnostic service running these studies (42,43).

PSG is highly useful in diagnosing CSA and hypoventilation syndromes, including monitoring response to treatment and titrating bilevel or positive airway pressure therapies. PG is less validated in these disorders and is not considered particularly useful for the screening or diagnosis of CSA (44). Therefore, any measurement technique less complex than PSG cannot be recommended for an indubitable diagnosis of any respiratory sleep disorder other than OSAHS at the present time.

Screening adult populations for respiratory sleep disorders

The definition of sleep disordered breathing depends on the AHI and ranges from 9% to 24% in the general adult population. The prevalence will change with age and differs between men and women (2).

EDS which occurs at least 3 days a week has been found to occur in 4-20.6% of the population. Severe EDS occurs in 5% of the population and men and women are equally affected (45).

History and clinical assessment are extremely important in attaining this diagnosis but require objective verification through overnight studies.

With regard to OSAHS, the commonest respiratory sleep disorder, several algorithms have been developed to predict its presence in patients presenting with suggestive complaints. These include utilising a combination of clinical variables such as BMI, neck circumference, jaw structure, snoring, reports of nocturnal breathing disturbances and the presence of hypertension. The sensitivity of these approaches is high (78-95%) but the specificity tends to be low (41-63%) (46).

Screening questionnaires for OSAHS have been utilised for some time. A recent meta-analysis of these questionnaires included 10 studies with a total of 1,484 subjects (47). Four studies utilised the Berlin questionnaire (48), two studies the Wisconsin Sleep Questionnaire (49), one study the STOP questionnaire (50) and one the STOP-BANG questionnaire (51). Four studies on referred patients gave a pooled sensitivity of 73% (95% CI, 66-78%) for the diagnosis of OSAHS and a pooled specificity of 61% (95% CI, 55-67%). Six studies on patients without a history of sleep disorders revealed a pooled sensitivity of 77% (95% CI, 73-80%) for detecting OSAHS and a pooled specificity of 53% (95% CI, 50-57%) (47).

Additional questionnaires have been devised over the last two decades to evaluate both sleepiness and quality of life in OSAHS. Whether they are applicable to any of the other respiratory sleep disorders discussed has not been explored. These include the Functional Outcomes of Sleep Questionnaire (52), the Calgary Sleep Apnoea Quality of Life Index (SAQLI) (53), the short SAQLI (54), the SSS (55)

and the ESS (30) as discussed above.

In terms of objective assessment and screening of large populations, Type III and IV device studies most likely represent the way forward. PG or Type IV device studies should always be conducted in conjunction with a comprehensive sleep evaluation. Results are improved if there is a high pre-test probability of sleep disordered breathing. At present, limited device studies (i.e., without EEG and out of hospital) are not indicated if the patient has significant co-morbidities, other associated sleep disorders, e.g., disorders of central hypersomnolence or an inability to use equipment despite education (56). Raw data requires manual scoring or editing, particularly if automated scoring is also available. Diagnostic accuracy can be questionable at times. Currently there are issues with scoring respiratory events using PG and even with home nasal pressure traces, as discussed above. The AASM criteria for PSG are not applicable to scoring respiratory events on PG.

Pulse oximetry is useful but presents some difficulties. At the present time there is no internationally standardised technical specification or standardisation of signal processing in oximeters with the minimum standard criteria set by the AASM comprising a sample rate of 25 Hz with an average of three values (6) and a resolution of 0.1% (57). The current desaturation definitions usually revolve around a decrease of >4% from baseline Sp02. However, there is no unanimity in terms of what constitutes a normal or abnormal desaturation index. Additional limitations of pulse oximetry include potential problems with blood flow and haemoglobinopathies, tissue optics in the very obese and an inability to detect other forms of sleep disordered breathing. Movement artefact needs to be taken into account and there can be significant measurement inaccuracies of ±2% in Sp02 (58).

Currently there is no data to suggest that oximetry is a useful tool in screening for CSA or disorders of hypoventilation.

Summary

Diagnostic approaches to respiratory sleep disorders are reasonably straightforward, but do require a combination of subjective and objective sleep assessment as well as a degree of clinical acumen when it comes to assessing severity and management options.

Monitoring and measuring respiration during sleep has undergone many advances in the last 40 years in respect of quality and validity, largely with respect to OSAHS. The technology has allowed for the recognition of other disorders of respiration during sleep including CSA and hypoventilation syndromes. Diagnosing respiratory sleep disorders on clinical features alone is limited. Despite the improvement in our diagnostic standards and recognition of sleep disordered breathing, many limitations still need to be overcome. Many researchers in the field continue to push the boundaries in respect of simplifying diagnosis and enhancing screening for sleep disordered breathing in large populations. At present, these approaches require further validation.

The most common disorder of respiration during sleep, OSAHS, can potentially be easily suspected and diagnosed in general practice with referral in difficult cases to secondary or tertiary specialised units. Since OSAHS affects 5% of the population in industrialised countries, it is as common as asthma (59) and if all forms of sleep disordered breathing are considered together they are as common as COPD (60). Just as the diagnosis and management of hypertension has devolved into general practice, then both subjective and objective assessments of OSAHS have the potential of being devolved into primary care.

This is unlikely to be true of the other sleep related breathing disorders at the present time. Unfortunately, there is still some way to go with respect to validating and ensuring that diagnoses are appropriate by "non-experts" prior to initiation of treatment. However, this is not an insurmountable task. The cost-effectiveness of simplified diagnostic tools with good sensitivity and specificity may eventually drive this approach and help to determine policy at governmental levels.

Respiratory sleep disorders are important, common conditions within our community. They pose a significant health burden and are frequently associated with other resource draining co-morbidities. Sleep disordered breathing can be diagnosed reasonably readily although management is not always straightforward.

Acknowledgements

Footnote

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Epidemiological aspects of obstructive sleep apnea

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Abstract: Obstructive sleep apnea (OSA) is probably the most common respiratory disorder, with recent data from the United States and Europe suggesting that between 14% and 49% of middle-aged men have clinically significant OSA. The intimate relationship between OSA and obesity means that its prevalence will only increase as the global obesity epidemic evolves. At an individual level, OSA leads to a significant decrease in quality of life (QOL) and functional capacity, alongside a markedly increased risk of cardiovascular disease and death. Emerging data also suggest that the presence and severity of OSA and associated nocturnal hypoxemia are associated with an increased risk of diabetes and cancer. At a societal level, OSA not only leads to reduced economic productivity, but also constitutes a major treatable risk factor for hypertension, coronary artery disease (CAD) and stroke. This article addresses OSA from an epidemiological perspective, from prevalence studies to economic aspects to co-morbidity.

Keywords: Sleep apnea; cardiovascular; economic; obesity

Introduction

Obstructive sleep apnea (OSA) is a highly prevalent disorder, characterised by recurrent episodes of upper airway obstruction occurring during sleep, and associated with recurrent cycles of desaturation and re-oxygenation, sympathetic over-activity and intra-thoracic pressure changes, leading to fragmentation of sleep and consequent daytime fatigue and sleepiness (1). OSA is associated with decreased quality of life (QOL), significant functional impairment, and increased risk of road traffic accidents. However, the relationship of OSA with medical co-morbidity, and in particular cardiovascular co-morbidity, is perhaps the area of most interest from a medical perspective (2). This review will discuss several epidemiological aspects of OSA, namely its prevalence, its close relationship with obesity, and its economic impact, alongside a discussion of population-level, epidemiological data evaluating the impact of OSA on cardiovascular and metabolic disease, and its emerging association with cancer.

Prevalence of OSA

When defined as repetitive upper airway obstruction during sleep, OSA is a very common disorder. Sleep apnea severity is usually assessed by the apnea-hypopnoea index (AHI) which is the number of complete (apneas) or incomplete (hypopneas) obstructive events per hour of sleep. Defining OSA as an AHI ≥5 events/hour in the Wisconsin Sleep Cohort, the prevalence of OSA was 24% in men and 9% in women aged 30-60 years of age (3). The prevalence of OSA with associated excessive daytime somnolence is approximately 3% to 7% in adult men and 2% to 5% in adult women (4). Prevalence estimates don't vary significantly worldwide suggesting that OSA is as common in the developing world as in western society (5).

As will be discussed in greater detail below, OSA has a particularly intimate association with obesity (4). Therefore, as the global obesity epidemic unfolds, it may be expected that the incidence and prevalence of sleep disordered breathing will increase in parallel with this. When data from the Wisconsin sleep cohort study were re-examined with adjustment for current levels of overweight and obesity, a marked increase in the prevalence of OSA was observed (6). Based on these data, 34% of men and 17.4% of women between the ages of 30-70 would be expected to have an AHI ≥5, which would be associated with excessive daytime

sleepiness in 14% of men and 5% of women. Emerging data from Europe suggest that the societal prevalence of the disorder may be even greater when modern diagnostic techniques are used: a community-based Swiss study of over 2,000 subjects diagnosed moderate-severe OSA (i.e., an AHI ≥15) in 23.4% and 49% of female and male subjects, respectively (7), potentially alarming findings which need to be confirmed in other studies and other population groups.

OSA and obesity

Obesity, especially central adiposity, is consistently recognised as one of the strongest risk factors for OSA. Given the worsening modern pandemic of obesity in western society, the prevalence of OSA is likely to increase further (4). In the Wisconsin Sleep Cohort study, weight gain over a 4-year period was an important predictor of OSA progression; a 10% increase in body weight conferred a 32% increase in AHI and a 6-fold increase in the risk of developing moderate-severe OSA (8). In the Sleep Heart Health Study, a multi-centre epidemiologic cohort study of cardiovascular correlates of OSA in middle-aged and older Americans, weight gain of 10 kilograms over a 5-year period conferred a 5.2- and 2.5-fold increase in the likelihood of increasing the AHI by 15 events per hour in men and women respectively (9). OSA is present in 41% of patients with a body mass index (BMI) greater than 28 and the prevalence can be as high as 78% in patients referred for bariatric surgery (10,11).

OSA is marked by repetitive collapses of the upper airway during sleep that occur due to reduced airway dilator muscle tone. Obesity may alter the normal upper airway mechanics and contribute to the pathophysiology of OSA in a number of ways (4). Parapharyngeal fat deposition can result in a reduction in calibre and a change in shape of the upper airway promoting collapsibility (12,13). Obesity is associated with a reduction in lung volumes, especially functional residual capacity, contributing to decreased tracheal tug, a decrease in upper airway size and increased airflow resistance (14). Leptin is a hormone produced by adipocytes in proportion to their triglyceride content and involved in the suppression of appetite (15). However, it also acts on the central respiratory centres to stimulate ventilation and leptin deficiency has been associated with hypoventilation (16,17). Obesity is characterised by central leptin resistance and there is blunting of the response to hypercapnia leading to worsening of hypercapnia and impairment of arousal from sleep during apneas (18).

It is postulated that the interaction between OSA and obesity is not unidirectional, and that OSA may also have an impact on the pathogenesis of obesity (19). It is well recognized that weight gain may be preceded by the onset of OSA symptoms (20), and reduced physical activity with an associated reduction in energy expenditure due to excessive daytime somnolence in OSA can promote weight gain (21). Calorie intake in OSA patients may also be higher (22). Sleep deprivation is a feature of OSA and sleep curtailment is linked with obesity, particularly in pediatric populations (23), potentially through alterations in the hormonal regulation of diet. Finally a number of studies have demonstrated elevated leptin levels in OSA patients compared to weight-matched controls and a decrease in leptin levels following continuous positive airway pressure (CPAP) therapy (24,25), suggesting that OSA may influence leptin metabolism independently of changes in weight (26).

Weight loss should be recommended for all overweight or obese patients with OSA, as it may confer not only a benefit in reducing OSA severity, but also a positive impact on other obesity-related diseases such as type 2 diabetes mellitus (T2DM). Patients with more severe OSA derive more benefit from weight loss than those with milder disease (27). Several studies have explored weight loss as a therapeutic option for OSA and approaches evaluated include behavioral methods (dietary modification and exercise), pharmacological methods and bariatric surgery (28). The results for behavioral modification have been mixed. Johansson reported a significant reduction in AHI to such an extent that 17% were cured, no longer requiring CPAP (27). Results from other studies showed no change in AHI however (29,30). Sibutramine, an oral anorexiant, has been evaluated in a number of studies in OSA patients, but any positive benefit it may have has now been superseded by concerns related to increased cardiovascular morbidity (nonfatal myocardial infarction and stroke) associated with its use (28).

Studies on the effects of bariatric surgery appear promising. In a Swedish case-control longitudinal study of more than 3,400 obese patients, the average fall in BMI was -9.7 ± 5 kg·m^{-2} compared to 0 ± 3 kg·m^{-2} in the control group (31). There was also a marked improvement in OSA symptoms and a lower 2-year incidence of T2DM and hypertriglyceridemia. Laparoscopic adjustable gastric banding in a cohort of severely obese patients with moderate to severe OSA resulted in significant weight loss and a decrease in AHI when evaluated at 17.7 ± 10.0 months after surgery (32). However, the majority of patients will still have residual OSA after surgery and care should be taken to ensure CPAP is not inappropriately discontinued (33,34).

OSA and age

The prevalence of OSA increases with age in adults (5). This age-related increase in prevalence may be attributable to parapharyngeal fat deposition, lengthening of the soft palate and changes in other anatomic parapharyngeal structures (35). The Sleep Heart Health Study demonstrated that the prevalence of OSA plateaued after the age of 60 years (36), and the increased risk of all-cause and cardiovascular mortality associated with OSA is mainly limited to middle-aged adults, especially men (37). Some researchers have suggested that the mortality risk with sleep apnea may even decrease in the elderly as a result of preconditioning cardioprotective adaptations to chronic intermittent hypoxia (38). Elderly patients with and without excessive daytime somnolence may represent different phenotypes that explain the conflicting data on mortality risk with increasing age in OSA; the presence of concomitant excessive daytime somnolence in OSA patients may increase the risk of mortality whereas the same severity of OSA without symptoms may not (39).

OSA and sex

It is well recognized that there is a higher prevalence of OSA in men than women, with most population-based studies demonstrating a 2- to 3-fold higher prevalence of OSA in men (4). Men are also more likely to be referred for clinical assessment for OSA (40), perhaps because physicians appear to have a higher index of suspicion for considering the disorder in men. This tendency may contribute to the underdiagnosis of OSA in women in clinical practice, a bias which may be compounded by the fact that women often do not present with the classical symptoms of OSA (loud snoring, witnessed apneas and excessive daytime somnolence) but instead may complain of poor energy levels and fatigue (41). Furthermore, the female bed partners of male patients may be more likely to perceive and report snoring or nocturnal breathing abnormalities than male bed partners of female patients.

Sex hormones may also play an important role in the pathogenesis of OSA. OSA is more prevalent in post-menopausal women than pre-menopausal women, and hormone replacement therapy in post-menopausal women may protect against the disorder (42,43).

Genetic aspects of OSA

Given its complexity as a disorder with multiple predisposing factors, the likelihood of a single governing genetic factor causing OSA is extremely low. That said, it is thought that up to 40% of the risk of OSA is genetically predisposed (44). The prevalence of OSA in first-degree relatives of patients with OSA ranges from 22-84% with an odds ratio (OR) of a first-degree relative having OSA ranging from 2 to 46 (45).

Anatomic risk factors for OSA, such as obesity and upper airway soft tissue structure, demonstrate familial aggregation. Obesity often persists from early life to late middle age and twin studies on Caucasian populations demonstrate a heritability estimate of 57-86% for the trend of BMI from early adulthood to late middle age (46). A case-control study in a Scottish cohort identified a strong familial component to OSA and suggested that differences in facial structure were more important than obesity in this regard (47). The lateral pharyngeal wall volume, tongue volume and total upper airway soft tissue volume have a significant level of heritability after adjusting for sex, age, ethnic background, craniofacial properties and neck fat deposition.

A number of candidate gene associations have been investigated in OSA including different alleles for apolipoprotein E4 (ApoE4), tumour necrosis factor (TNF), and angiotensin-converting enzyme (ACE) but only one TNF polymorphism (TNFA rs1800629) was significantly associated with OSA under an allele frequency model (48).

Economic aspects of OSA

OSA has a significant economic impact on healthcare systems and society. OSA-related healthcare cost includes the direct costs of OSA diagnosis and treatment and the indirect costs of associated conditions (obesity, diabetes) and sequelae (cardiovascular disease, depression). Patients with sleep disorders are less productive workers than those without sleep disorders and have a higher level of absenteeism and decreased productivity at work due to fatigue (49). Occupational injuries are also more common in patients with OSA (50).

Several studies have shown that QOL is adversely affected in patients with OSA and that it improves with therapy (51-53). The bed partner of a patient with untreated OSA may also suffer from disrupted sleep because of the patient's snoring, gasping or witnessed apneas. The QOL of bed partners also improves significantly when OSA is treated (54,55).

Untreated patients with OSA are at an increased risk of motor vehicle accidents (MVAs). Patients with OSA have

a 2- to 10-fold increased risk of MVAs and the severity of accident may be greater compared to controls (56,57). OSA-related MVAs in the USA were estimated to involve 800,000 drivers and cost $15.9 billion annually (58). Treatment with CPAP therapy reduces this risk in compliant patients to that of controls (59).

The results of cost-effectiveness analyses support the utilization of CPAP therapy in patients with moderate to severe OSA relative to other commonly accepted medical interventions. It is estimated that it costs $2,000—11,000 per quality-adjusted life year (QALY) over 5 years to treat moderate to severe OSA in a sleep clinic population compared to $5,000—19,000 for breast cancer screening and $10,000—57,000 for hypertension control (5).

Epidemiology of co-morbidity in OSA

OSA and cardiovascular disease

OSA and hypertension

The most robust evidence supporting an independent role for OSA in promoting adverse cardiovascular outcomes is to be found in studies addressing its relationship with hypertension (1). Following on from a number of studies where snorers were more likely to also have hypertension than might otherwise be expected (60), a series of clinical and epidemiological studies identified a dose-response relationship between OSA severity and the likelihood of prevalent hypertension (61). For example, a cross sectional analysis of over 6,000 North American subjects enrolled in the community-based Sleep Heart Health Study showed that subjects with severe sleep disordered breathing had an OR of 1.37 [95% confidence interval (CI): 1.03-1.83] for prevalent hypertension following adjustment for confounding factors when compared to those with no OSA (62). Recent European data underline this association, and suggest that intermittent hypoxemia may be a key factor contributing to co-existent hypertension—among 11,900 participants in the multi-national the European Sleep Apnea Cohort (ESADA) study, OSA severity indices, and in particular the oxyhemoglobin desaturation index, were strong independent predictors of hypertension (63).

Subsequent longitudinal studies have provided convincing evidence that OSA contributes to an increased risk of clinically-relevant hypertension. Landmark findings among participants in the Wisconsin Sleep Cohort study demonstrated a strikingly increased propensity for the development of incident hypertension in subjects with sleep disordered breathing (64). In an analysis of 709 subjects, the presence severe OSA at enrollment conferred a nearly threefold risk of being diagnosed with hypertension over a four-year follow-up period, independently of the effects of age, obesity and smoking history. More recent data from Spain have confirmed this relationship (65).

OSA and coronary artery disease (CAD)

Data from clinic and community based studies generally suggest that CAD is highly prevalent in OSA cohorts (66), and *vice versa* that subjects with CAD are more likely to also have sleep disordered breathing, even allowing for the impact of obesity and other confounding factors (67). For example, in over 6,000 participants in the Sleep Heart Health Study, the burden of self-reported CAD among these subjects increased from 9% in the lowest AHI quartile to 19% in the highest, a relationship that survived adjustment for confounding variables (68).

It is less clear if the presence and severity of OSA actually serve as independent predictors of subsequent CAD, however. While data from relatively small, but well-conducted studies of hospital patients suggest a robust dose-response relationship between the two (69), these data have not been reproduced to the same degree at a population level. A relatively modest relationship between OSA and CAD incidence was seen in a community-based study of 4,422 (56.4% female) North American subjects followed for a median of 8.7 years (37). Within this cohort, severe OSA predicted an increased risk of developing symptomatic CAD, but only in men aged 70 or less (HR 1.68; 95% CI: 1.02-2.76). However, another analysis of this cohort has shown severe OSA to be an independent predictor of death, and in particular death related to CAD (70). While this relationship was again strongest in men under 70 years of age (adjusted HR 2.09; 95% CI: 1.31-3.33), it was nonetheless seen across the entire study population (adjusted HR 1.46; 95% CI: 1.14-1.86), with similar findings seen at eighteen year follow-up in the Wisconsin Sleep Cohort study (71).

Were OSA playing a causative role in driving cardiovascular morbidity and mortality, CPAP therapy might be expected to lead to measurable reductions in adverse cardiac outcomes. In a study of 1,652 subjects attending a Spanish sleep laboratory, cardiovascular outcomes were assessed over an average of 10.1 years (72). Those with untreated severe OSA (14.2%) were more likely to die of cardiovascular disease (adjusted OR 2.87; 95% CI: 1.17-7.51) or experience cardiovascular morbidity (adjusted OR

3.17; 95% CI: 1.12-7.51) compared with healthy non-apneic subjects. In contrast, no significant increased risk of cardiac death was seen in OSA patients who had been successfully commenced on CPAP (adjusted OR 1.05; 95% CI: 0.39-2.21). As yet there are no published data from large-scale randomised trials in this area, although a number of potentially important studies with this goal are ongoing.

OSA and heart failure

Obstructive sleep apnea is highly prevalent in heart failure patients, and is independently associated with increased prevalence of clinically overt heart failure (73). In a cross sectional analysis of the Sleep Heart Health study, severe OSA was associated with increased likelihood of prevalent heart failure, with an adjusted OR of 2.20 (95% CI: 1.11-4.37) in the highest AHI quartile (68). Longitudinal follow-up in the same cohort identified a strong relationship between incident heart failure and AHI in men but not women (37). Following adjustment for demographic, anthropometric and clinical factors and medication usage, male participants with severe OSA at baseline had a 58% increased risk of developing heart failure over nearly nine years of follow-up. When present, OSA appears to lead to increased risk of mortality in heart failure cohorts (74).

OSA and cardiac arrhythmia

Subjects with OSA are at increased risk of atrial fibrillation (AF); in a nested case control study involving 566 participants in the Sleep Heart Health study, severe sleep disordered breathing was associated with a four-fold odds of prevalent AF, following adjustment for age, gender, obesity and the presence of CAD (adjusted OR 4.02; 95% CI: 1.03-15.74) (75). The presence of OSA predicts an increased likelihood of risk factors for AF, such as increased left atrial diameter (76), and furthermore is associated with an increased risk of recurrence of AF and failure of chemical cardioversion measures (77,78). Moreover, recurrence of AF becomes less likely with the initiation of CPAP treatment (77). Sleep disordered breathing is also an independent predictor of ventricular arrhythmia, particularly in subjects with heart failure (75).

OSA and cerebrovascular disease

Sleep disordered breathing is significantly more common in patients who have had a stroke or transient ischaemic attack (TIA) than in the general community, occurring in between 32-63% of stroke patients, and is associated with increased mortality and worse functional outcomes in these patients

(79,80). Furthermore, cerebrovascular disease prevalence appears to increase with increasing OSA severity. Among 6,089 subjects in the Sleep Heart Health Study, 2.7% in the lowest AHI quartile had a prior stroke or TIA, compared with 5.3% in the most severe quartile, a relationship which persisted following adjustment for relevant confounders (adjusted OR 1.58; 95% CI: 1.02-2.46) (68). A number of prospective studies suggest that the presence and severity of sleep disordered breathing predicts incident stroke (81-84). For example, among 5,422 North American subjects followed for a median of 8.7 years, those in the most severe AHI quartile had an adjusted HR 2.86 (95% CI: 1.1-7.4) for stroke over the study period (83) (*Table 1*).

OSA and metabolic disease

The complex relationship between OSA, obesity and metabolic disease, and in particular insulin resistance, glucose intolerance and T2DM is the subject of a forthcoming review in *Journal of Thoracic Disease*, and will not be discussed in detail here. Briefly, it does appear that OSA severity has a direct bearing on metabolic health. For example, in a large study of over 6,000 subjects attending European sleep laboratories, severe OSA was associated with an almost twofold increase in likelihood (adjusted OR 1.87) of concomitant T2DM, even following adjustement for the confounding effects of obesity, age and other variables (85). Moreover, diabetic patients with severe OSA within this cohort had higher HbA1c levels, and were more likely to have poorly controlled T2DM than non-apneic diabetics. Similarly, in an historical cohort study of 8,678 Canadian sleep clinic patients, followed for a median time period of 67 months, a diagnosis of severe OSA conferred a 37% increase in risk for incident T2DM, following statistical adjustment for confounding factors (86).

Insulin resistance and glucose intolerance also appear to be more common in OSA populations. In a large North American community-based cohort, severity of nocturnal hypoxemia and the presence of at least moderately severe OSA were both associated with an increased odds of insulin resistance (87), while a study of over 5,000 non-diabetic European sleep clinic patients found that HbA1c levels increased in parallel with AHI, irrespective of the influence of obesity (88).

Once again, the ability of CPAP to make a meaningful difference to metabolic health in patients with OSA remains unproven. A number of well-conducted, if relatively small, randomised trials have generally failed to identify anything

Table 1 Selected population studies of associations between OSA and cardiovascular disease

Authors	Participants	Study design	Outcome measures	Findings
OSA and hypertension				
Nieto et al. (62)	6,132 subjects Community based	Cross sectional	Relationship of OSA severity with prevalent hypertension	Severe OSA associated with increased odds of prevalent hypertension (AOR 1.37)
Tkacova et al. (63)	11,911 subjects Clinic based	Cross sectional	Hypertension prevalence	AHI and ODI both independently predict prevalent hypertension; Stronger relationship between ODI and hypertension
Peppard et al. (64)	709 subjects Community based	Observational 4-8 years follow-up	Hypertension incidence	Increased hypertension incidence with increasing OSA severity; AOR 2.89 for AHI >15 vs. AHI 0
Marin et al. (65)	1,889 subjects Clinic based	Observational 12.2 years median follow-up	Hypertension incidence; Analysis stratified by CPAP prescription and compliance	OSA associated with increased risk of hypertension; Increase in incidence abrogated by CPAP use
OSA and coronary artery disease				
Gottlieb et al. (37)	1,927 men 2,495 women Community based	Observational 8.7 years median follow-up	CAD disease incidence	No relationship between OSA severity and CAD in women; HR 1.68 in men aged 40-70 with AHI ≥30
OSA and stroke				
Redline et al. (83)	5,422 subjects Community based	Observational 8.7 years median follow-up	Stroke incidence	HR 2.86 in men in highest AHI quartile; Non-significant relationship with OSA severity in women
OSA and cardiovascular mortality				
Young et al. (71)	1,522 subjects Community based	Observational 18 years follow-up	Cardiovascular mortality	HR 5.2 with severe OSA in subjects who did not use CPAP
Marin et al. (72)	1,651 subjects Clinic based	Observational 10.1 years mean follow-up	Cardiovascular mortality; Analysis stratified by CPAP use	HR 2.87 for fatal cardiovascular events with severe OSA; CPAP use associated with incidence similar to controls

AHI, apnoea-hypopnoea index; AOR, adjusted odds ratio; ODI, oxyhemoglobin desaturation index; CAD, coronary artery disease; HR, hazard ratio; OSA, obstructive sleep apnea; CPAP, continuous positive airway pressure.

more than a minor impact from CPAP therapy on a range of metabolic variables (89-92).

OSA and malignancy

Sleep disordered breathing generates a molecular environment which is certainly markedly pro-atherogenic (93), but could also potentially promote the development of cancer. A recognition that OSA causes tissue hypoxia, systemic inflammation, oxidative stress and immune dysregulation (93), all factors associated with oncogenesis, lead a number of investigators to examine the effects of intermittent hypoxia in a rodent model of maligant melanoma. Data from these animal models suggested that exposure to IH may increase tumor progression, and appears to promote tumor metastasis (94,95).

A number of longitudinal studies have since suggested that an association between OSA, nocturnal hypoxemia and cancer may not be confined to rodents living in laboratories. In an analysis of 1,522 subjects enrolled in the community-based Wisconsin Sleep Cohort, severe sleep disordered breathing was associated with an almost five-fold risk of cancer death (96). Similarly, in a cohort of nearly 5,000 Spanish patients attending sleep clinics, severity of nocturnal hypoxemia predicted incident cancer, even following rigorous adjustment for confounding variables (97). Conversely, a large retrospective cohort study of Canadian sleep found that any relationship between OSA and cancer incidence could be attributed to conventional, established risk factors for malignancy (98); however, even within this population an independent association was seen between smoking-related cancer and severity of nocturnal hypoxemia. To date, there are no published data examining the effect of CPAP therapy on cancer outcomes, but potentially ground-breaking clinical trials are ongoing in this area.

Conclusions

OSA is an increasingly common disorder, with a particularly intimate relationship with obesity. OSA leads to impaired QOL, an increased risk of MVAs and impaired workplace performance. Furthermore, it contributes to development of clinically overt cardiovascular disease, and may also lead to increased risk of metabolic disease and cancer. Consequently, OSA represents a significant, evolving public health challenge in both the developed and developing world.

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Established vascular effects of continuous positive airway pressure therapy in patients with obstructive sleep apnoea—an update

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Abstract: The aim of this review was to summarize the current data from randomised controlled trials (RCTs) on vascular effects of continuous positive airway pressure (CPAP) therapy in patients with obstructive sleep apnoea (OSA). There is good evidence from RCTs that CPAP lowers blood pressure (BP) to a clinically significant amount. The effect seems to be dependent on the hours of nightly CPAP usage. Data from RCTs have also proven a beneficial effect of CPAP on measures of vascular function such as endothelial function and arterial stiffness. However, there is still a lack of evidence from RCTs proving that CPAP reduces vascular events and mortality.

Keywords: Obstructive sleep apoea (OSA); endothelial function; continuous positive airway pressure (CPAP); arterial hypertension

Introduction

In the last thirty years a large body of observational and epidemiological studies has described an association between obstructive sleep apnoea (OSA) and increased incidence of vascular morbidity and mortality (1-4).

The findings of physiological studies and clinical trials have established that pathophysiological consequences of OSA such as sympathetic activity, intermittent hypoxia and oxidative stress as well as intrathoracic pressure swings play a key role in the development of vascular dysfunction in patients with OSA. Therefore a biological plausibility for a causal association between OSA and cardiovascular events can be assumed (5-7).

Continuous positive airway pressure (CPAP) is the gold standard therapy for OSA and has been proven to reduce daytime sleepiness and enhance quality of life in patients with OSA (8). Moreover, several observational studies suggested an association between CPAP therapy and reduced cardio- and cerebrovascular morbidity and mortality in patients with OSA. The mechanisms involved

in this beneficial effect of CPAP on the vascular system likely include a reduction of blood pressure (BP) and improvement of vascular function (9-12).

The aim of this review was to summarize the currently established effects of CPAP on BP and vascular function in patients with OSA.

CPAP effects on BP

One major mechanism underpinning the association between OSA and cardiovascular disease is likely to be sustained arterial hypertension, and this association may possibly be enhanced by frequent nocturnal acute BP rises. The repetitive episodes of obstructive apnoeas and hypopnoeas are often associated with arousals and intermittent hypoxia, both of which lead to increased sympathetic nervous system activity and consequent considerable transient increases in arterial BP which can be as high as 80 mmHg. CPAP treatment has been shown to not only effectively abolish apnoeas, hypopnoeas and oxygen desaturations, but also to prevent arousals and, thus obviate acute BP rises (7,13).

Randomised controlled trials (RCTs) in patients with moderate to severe OSA

Several RCTs looking at the effect of CPAP on ambulatory BP have been conducted in the past decades; the results of these trials have established that CPAP treatment of patients with moderate to severe symptomatic OSA lowers BP to a variable extent. Most of the trials reported a reduction in BP of between 2 and 10 mmHg after several weeks of CPAP therapy (13,14). The effect of CPAP therapy on BP seems to depend on the severity of the sleep disordered breathing, the presence of daytime sleepiness, the extent of obesity, BP values before CPAP treatment and hours of nightly CPAP use (15-17). The findings of recent studies suggest that, in symptomatic patients, the beneficial effects of CPAP on BP are found mainly in those who show good adherence to treatment (e.g., at least 4 h per night), and this may also be true for patients without overt daytime sleepiness (18,19).

RCTs in patients with mild OSA

A common question faced by sleep physicians is at which level of disease severity patients with OSA should be treated. There is particular uncertainty about the need and the effectiveness of treatment in mild cases of OSA, especially when treatment would be prescribed to reduce cardiovascular risk.

Barnes et al. (20) performed a RCT in 28 patients with mild OSA [mean apnoea hypopnoea index (AHI) of 12.9/h], who underwent eight weeks of CPAP treatment vs. an oral placebo tablet. Compared to placebo, CPAP did not improve Epworth Sleepiness Scale (ESS) as a measure of daytime sleepiness. No benefit of CPAP compared to placebo was found on 24 h BP.

Newer data from a RCT conducted by Weaver et al. (21) evaluate the efficacy of CPAP treatment to improve functional status assessed by the Functional Outcomes of Sleep Questionnaire (FOSQ) in sleepy patients with mild and moderate OSA. A total of 239 patients with a mean AHI of about 13/h were randomized to CPAP treatment or placebo. After eight weeks CPAP treatment significantly improved the functional outcome of patients with mild OSA and there was a significant change in daytime diastolic BP values from baseline by –1.93 mmHg (95% CI, –3.8 to 0.0; P=0.048) between the two groups (21).

Both RCTs were not powered adequately to investigate the treatment effect on BP. Thus, further trials are needed to definitely clarify if patients with mild OSA benefit from CPAP treatment in terms of BP reduction.

RCTs in patients with oligo-symptomatic OSA

Half of all individuals with moderate to severe OSA do not report excessive sleepiness (22,23). An association between oligo-symptomatic OSA and cardiovascular disease has not been established so far, and it is unclear whether CPAP treatment results in improved vascular risk in this group of patients.

Recently, Barbé and colleagues (24) published the data of a RCT which evaluated the effect of CPAP treatment on the incidence of hypertension and cardiovascular events in a cohort of non-sleepy patients with OSA. The 725 consecutive patients with an AHI of ≥20/h and an ESS score of ≤10 were enrolled. In this cohort of OSA patients without daytime sleepiness, CPAP treatment did not result in a statistically significant reduction in the incidence of hypertension and cardiovascular events compared to usual care after a median follow-up of 4 years. However, there was some evidence that patients who were highly compliant with CPAP (>5.6 h/night) benefited from this treatment as a reduction of BP and cardiovascular events was observed in such patients (24).

In the Multicentre Obstructive Sleep Apnoea Interventional Cardiovascular (MOSAIC) trial 391 patients with oligo-symptomatic OSA were randomised to 6 months of auto-adjusting CPAP therapy or standard care. The investigators demonstrated that CPAP treatment significantly improved subjective daytime sleepiness (adjusted treatment effect on ESS –2.0; 95% CI, –2.6 to –1.4; P<0.0001). However, this positive treatment effect on symptoms was not accompanied by a reduction in calculated vascular risk or BP (25).

The findings of the MOSAIC study were confirmed by a meta-analysis published by Bratton et al. (26), in which the individual data of 1,206 patients from four RCTs have been evaluated. Although CPAP treatment reduced OSA severity and sleepiness in minimally symptomatic patients, overall it did not to have a beneficial effect on BP, except in those patients who used CPAP for >4 h/night (26).

RCTs in patients with resistant hypertension

OSA has been proposed as a risk factor for resistant hypertension, which is defined as repeatedly measured BP >140/90 mmHg despite the use of three or more antihypertensive drugs of different classes. It has been estimated that more than 70% of patients with resistant hypertension have OSA (27).

There are a number of recently published RCTs on the effect of CPAP on BP in patients with resistant hypertension

(28-30). In a first RCT conducted by Lozano *et al.* (29), 64 patients were randomised to receive CPAP added to conventional treatment or conventional medical treatment alone. They completed a follow-up after 3 months and patients who used CPAP >5.8 h showed a greater reduction than patients treated with standard medication in daytime diastolic BP −6.12 mmHg (95% CI, −1.45 to −10.82; P=0.004), 24-h diastolic BP 6.98 mmHg (95% CI, −1.86 to −12.1; P=0.009) and 24-h systolic BP −9.71 mmHg (95% CI, −0.20 to −19.22; P=0.046). Additionally, the number of patients with a dipping pattern significantly increased in the CPAP group compared to conventional medical treatment (51.7% *vs.* 24.1%, P=0.008) (29).

Pedrosa *et al.* (28) randomised 20 patients with resistant hypertension to standard antihypertensive treatment and 20 patients to antihypertensive treatment plus CPAP for 6 months. Daytime ambulatory BP decreased significantly in the group allocated to antihypertensive treatment plus CPAP compared to standard antihypertensive treatment alone; the difference between groups in systolic and diastolic BP was also significant (9.6±6.6 and 6.6±4.6 mmHg; P<0.05). Interestingly, in this trial there was no beneficial effect of CPAP on nocturnal BP. This may be explained partly by recurrent arousals induced by the repetitive BP measurements during the night masking any underlying benefit, or the possibility that resistant hypertension is a hyperadrenergic condition that itself leads to frequent arousals (28).

In the HIPARCO-trial (30), a Spanish multicentre RCT, 194 patients with resistant hypertension and an AHI of ≥15/h were randomised to CPAP in addition to standard antihypertensive treatment or antihypertensive medication alone. After 12 weeks of CPAP treatment a higher prevalence of nocturnal dipper pattern and a reduction of nocturnal riser pattern have been observed. The recovery of the nocturnal dipper pattern may be advantageous for long-term cardiovascular outcome as the presence of a non-dipping or rising BP pattern is recognised as an independent cardiovascular risk factor (31). Linear regression analysis showed a reduction of 1.9 mmHg (95% CI, 0.6 to 3.3) in systolic BP and 1.0 mmHg (95 % CI, 0.1 to 1.8) in diastolic BP for each additional hour of CPAP use (30).

Meta-analyses

The extent to which CPAP can reduce BP in OSA patients is still under debate. Up to date, numerous meta-analyses evaluated the effects of CPAP on BP. In the following we focus on three recently published meta-analyses summarizing the relevant RCT data on the effect of CPAP therapy on BP (32-34).

Schein *et al.* (32) reviewed 16 RCTs which included 1,166 OSA patients in total. The use of CPAP resulted in clinically relevant reductions of BP; CPAP treatment was associated with a reduction of systolic BP by 3.20 mmHg (95% CI, 1.72 to 4.67) and diastolic BP by 2.87 mmHg (95% CI, 0.55 to 5.18) (32).

A further meta-analysis by Montesi *et al.* (34) including 32 RCTs showed similar results. OSA patients treated with CPAP benefitted from significant reductions in systolic BP by 2.58 mmHg (95% CI, 3.57 to 1.59) and diastolic BP by 2.01 mmHg (95% CI, 2.84 to 1.18). Night-time systolic BP was the variable with the most prominent reduction after treatment with CPAP (4.09 mmHg, 95% CI, 6.24 to 1.94) (34).

In their recently published meta-analysis of 29 RCTs including 1,820 participants, Fava *et al.* (33) also observed a decreased systolic BP (2.6±0.6 mmHg) and diastolic BP (2.0±0.4 mmHg) in patients with CPAP treatment. As a result of their meta-regression analysis they concluded that patients with frequent apnoeic episodes may experience the largest benefit from CPAP therapy with regard to BP reductions; for each increase in AHI of 10/h the systolic BP was predicted to decrease approximately 1 mmHg with CPAP treatment (33).

The relatively small treatment effects of CPAP on BP found in the meta-analyses may be related to methodological differences among the included trials, different study populations (e.g., sleepy and non-sleepy patients), sample sizes, study designs and the techniques used to measure BP (e.g., single time point, 24 h BP, beat-to-beat BP).

Clinical implications

Considering the recent RCT data, treatment with CPAP promotes small but clinically significant reductions in BP in individuals with OSA. Thus, a combined treatment including both antihypertensive medication and CPAP may be required in more severely hypertensive OSA patients. This combination is likely to be more effective in lowering both nocturnal and daytime BP than either treatment alone. The subsequent reduction in cardiovascular risk may be substantial, however this needs to be shown in a RCT (35,36).

CPAP effects on endothelial and vascular function

Endothelial dysfunction is an early marker of vascular

damage that precedes clinically overt vascular disease and is an important predictor of cardiovascular events. Early recognition of atherosclerotic changes and endothelial dysfunction may have an impact on risk stratification and thus influence the clinician's decision whether or not to aim for risk factor reduction in such patients. Evidence underpinning the association between OSA and impaired endothelial function and reduced endothelial repair capacity has been accumulating in recent years (37-41).

One well-described mechanism of endothelial dysfunction is the reduced bioavailability of endothelium-derived vasodilating factors such as nitric oxide (NO). Flow-mediated dilatation (FMD) of the brachial artery is currently the best-validated technique to non-invasively measure peripheral endothelial function. This method quantifies NO-mediated vasodilatation resulting from shear-stress mediated activation of endothelial NO synthase in response to an acute increase in luminal blood flow (42,43).

A possible underlying mechanism for endothelial dysfunction in patients with OSA seems to be a down regulation of endothelial NO synthase as a result of increased sympathetic activity, oxidative stress, excessive arterial wall shear stress caused by recurrent surges in BP during apnoeic events, increased endothelial cell apoptosis as well as increased levels of coagulation factors and cholesterol (37,44).

RCTs in patients with moderate to severe OSA

The first RCT investigating the impact of CPAP therapy on FMD in moderate to severe OSA by Ip and colleagues (45) resulted in a significant increase of FMD in the CPAP group after four weeks of therapy, whereas those on standard care showed no significant change (absolute between-group difference in FMD of 5.4%, P<0.001).

In a RCT conducted by Kohler *et al.* (18) a significant decrease in endothelial function (FMD) was observed after 1 week [–1.7% (95% CI, –2.8 to –0.6); P<0.002] and 2 weeks [–3.2% (95% CI, –4.5 to –1.9); P<0.001] of CPAP withdrawal in patients with moderate-severe OSA compared to continued CPAP use.

RCTs in patients with mild OSA

To date, there are no published data from RCTs on the effect of CPAP on endothelial function in patients with mild OSA.

RCTs in patients with oligo-symptomatic OSA

Recent data from the MOSAIC-trial, a multicentre RCT

evaluating the cardiovascular risk in 391 patients with minimally symptomatic OSA, CPAP treatment showed beneficial effects on endothelial function as assessed by FMD +2.1% (95% CI, 1.0 to 3.2; P<0.001). The improvement in FMD was larger in patients using CPAP for >4 h/night than in those who used it less (P<0.013) (46).

Meta-analysis

Between 2004 and 2013, 6 RCTs have been performed measuring FMD in patients with OSA before and after 2-24 weeks of CPAP treatment. RCTs evidenced that CPAP treatment improves endothelial function. Compared to the control group, CPAP therapy significantly increased FMD by 3.9% (95% CI, 1.9 to 5.8, P<0.0001) (47).

CPAP effects on arterial stiffness

Increased arterial stiffness is an early indicator of arterial disease. Augmentation index (Aix) and pulse wave velocity (PWV) are measures of arterial stiffness and independently predict cardiovascular events in high-risk populations (48). The shape of the pressure waveform of an artery provides a measure of arterial stiffness and can be assessed by the technique of pulse wave analysis (49).

RCTs in patients with moderate to severe OSA

Drager *et al.* (50) randomly assigned 24 patients with severe OSA without comorbidities to receive no treatment or CPAP for 4 months. After this period of CPAP treatment they found a significant decrease of arterial stiffness as assessed by PWV (10.4±1.0 *vs.* 9.3±0.9 m/s; P<0.001) (50).

In another RCT by Kohler *et al.* (14) a significantly decreased Aix from 14.5% to 9.1% was observed in patients with moderate to severe OSA after 4 weeks of CPAP treatment compared to sham CPAP. This considerable reduction is comparable in size to the effect seen after 12 weeks of exercise training in patients with coronary artery disease or after 6 weeks of eprosartan (600 mg daily) in patients with never treated arterial hypertension (14).

In contrast, Jones *et al.* (51) could not find a significant decrease of Aix (15.5%±11.9% *vs.* 16.6%±11.7%; P=0.08) in 43 patients with an AHI >15/h after 12 weeks of CPAP or sham-CPAP treatment in their RCT. An important limitation of this study and possibly the reason why there was no significant effect of CPAP on Aix in the latter study is the very low nightly CPAP usage of 3 h/night (51).

RCTs in patients with mild OSA

At present there are no data from RCTs evaluating the effects of CPAP therapy on arterial stiffness in patients with mild OSA.

RCTs in patients with oligo-symptomatic OSA

A recently published RCT assessed Aix by pulse wave analysis in 208 non-sleepy OSA patients who underwent 6 months of CPAP treatment or continued standard care. There was no statistically significant effect of CPAP on Aix observed (–1.4%; 95% CI –3.6 to 0.9; P<0.23). An explanation for the lack of an effect in this study may be that the population of patients were not only non-sleepy but also had milder OSA than in the other published RCTs (50,52). In addition, the study population had a higher age and higher proportion of patients with cardiovascular comorbidities than those of previous studies, both of which are well known to increase arterial stiffness and, thus, may have masked a positive effect of CPAP (46).

Meta-analysis

A recently published meta-analysis by Vlachantoni *et al.* (48) included 615 patients from 11 interventional studies and four RCTs. Overall significant improvements were observed in all indices of arterial stiffness after CPAP treatment.

Nevertheless, the potential beneficial effects of CPAP in reducing arterial stiffness in patients with mild OSA and the impact of CPAP adherence on the treatment effect should be explored in future studies (48).

CPAP effects on vascular events

Data from observational cohort studies suggest that OSA is associated with vascular morbidity and mortality (1,12). In contrast to these findings, Barbe and colleagues (24) as well as the investigators of the MOSAIC-trial (25), who analysed the effects of long-term CPAP therapy on cardiovascular risk in non-sleepy OSA patients could not establish a beneficial effect of CPAP treatment on cardiovascular events.

Thus evidence is needed from large RCTs to evaluate whether CPAP treatment is a useful therapy to prevent vascular events in patients with OSA. There is ongoing research in this field and data answering some of the open questions may soon be available (53).

Promising is the Sleep Apnea Cardiovascular Endpoints Study SAVE (NCT00733343), a multi-centre, open label, parallel, prospective, RCT that investigates the effects of CPAP treatment plus standard care versus standard care alone in 2,500 high risk subjects for CAD with moderate-severe OSA. The trial will determine the effects of CPAP treatment over a 2-7-year follow-up period on new cardiovascular events, including MI, stroke and cardiovascular death. The study is conducted in China, Australia, New Zealand, Spain and Brazil and a completion of this trial is announced for December 2015 (5,54).

Another large-scale multi-centre RCT (the Randomized Intervention with CPAP in Coronary Artery Disease and Sleep Apnoea-RICCADSA trial, NCT0051959) investigates patients with asymptomatic OSA and stable CAD. This study completed recruitment and included 511 patients with CAD undergoing planned percutaneous or surgical coronary revascularization and assesses whether CPAP treatment reduces the combined rate of new revascularization, MI, stroke and cardiovascular mortality over a follow-up period of 3 years (55).

ISAACC, another notable trial (CPAP in Patients With Acute Coronary Syndrome and OSA-trial, NCT 01335087) will include more than 1,800 OSA patients with a recent acute coronary syndrome (ACS) to clarify whether CPAP treatment reduces the rate of major cardiovascular events in patients with non-ST elevation or ST elevation ACS admitted to a coronary care unit during a 12-month follow-up (53,56).

The US National Institute of Health has funded three planning grants, the Heart Biomarker Evaluation in Apnea Treatment (HeartBEAT, NCT01086800), Best Apnea Interventions in Research (BestAIR, NCT01261390) and the Sleep Apnea in TIA/Stroke (SleepTight, NCT01446913) studies to evaluate design approaches for a large scale clinical trial of CPAP for cardiovascular risk reduction, including effectiveness of various recruitment strategies, methods for optimizing adherence, use of control treatments, intermediate endpoints most responsive to intervention and the use of oxygen as an alternative to CPAP (5).

Conclusions

Although numerous RCTs found clinically significant reductions of BP with CPAP treatment in patients with moderate to severe symptomatic OSA, CPAP indication is still debatable in patients with mild OSA or in patients without daytime sleepiness. Hence, OSA treatment must be tailored for each patient, based on metabolic and

cardiovascular risks and the willingness of patients to use CPAP on a nightly basis. Alternative or combined treatments are needed to reduce cardiovascular risk, particularly in minimally symptomatic patients, who are less likely to accept CPAP (57). The ongoing RCTs have to be awaited before CPAP therapy can be regarded as an effective treatment to protect from vascular morbidity and mortality (37).

Acknowledgements

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Insulin resistance, glucose intolerance and diabetes mellitus in obstructive sleep apnoea

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Abstract: Obstructive sleep apnoea (OSA) is a highly prevalent disorder, which conveys an increased risk of cardiovascular disease and death. Type 2 diabetes mellitus (T2DM), glucose intolerance and insulin resistance (IR) are common in subjects with OSA, but a shared intimate relationship with obesity makes discerning an independent link challenging. Nonetheless, mechanistic studies suggest that OSA could contribute to impaired glucose metabolism via the effects of sleep fragmentation, sympathetic excitation and intermittent hypoxia (IH) on pancreatic B-cell function, insulin sensitivity, and systemic inflammation. In particular, emerging data suggest that IH may have an important detrimental effect on adipose tissue function and inflammation. Similarly, data from population—and clinic-level studies suggest that OSA is independently related with the prevalence and incidence of T2DM and IR, and may also lead to worse glycaemic control in diabetics. However, the ability of continuous positive airway pressure (CPAP) therapy to make a meaningful impact on T2DM or IR remains uncertain. In this review we explore the available evidence linking OSA with IR, glucose intolerance and T2DM, and discuss potential pathobiological mechanisms by which sleep disordered breathing can affect metabolic health.

Keywords: Obstructive sleep apnoea (OSA); intermittent hypoxia (IH); insulin resistance (IR); diabetes mellitus; inflammation

Obstructive sleep apnoea (OSA) is a clinical syndrome characterized by repeated episodes of pharyngeal obstruction during sleep that lead to intermittent hypoxia (IH), sleep fragmentation and excessive daytime sleepiness. It is a highly prevalent disorder affecting about 14% of men and 5% of women and its prevalence is rapidly rising due to the strong association of OSA with obesity (1). The major health burden in OSA patients is an increased risk of cardiovascular diseases, such as systemic arterial hypertension, coronary artery disease, heart failure and stroke, an association which is corroborated by numerous large-scale epidemiological and prospective studies (2). Furthermore, there is increasing evidence of an independent association of OSA with metabolic dysfunction, and in particular with alterations in glucose metabolism. Subjects with OSA seem to be at greater risk of developing type 2 diabetes mellitus (T2DM), insulin resistance (IR) and metabolic syndrome (MetS), an association which seems to be at least in part irrespective of the degree of obesity. Indeed, OSA and obesity may exert synergistic negative effects on glucose metabolisms. However, studies in this field have yielded inconsistent results, and the impact of OSA treatment on glucose metabolism and metabolic dysfunction is also not clearly established.

In this article, we will review the current evidence evaluating potential links between OSA and T2DM, IR and

MetS, including the potential impact of continuous positive airway pressure (CPAP) treatment on these consequences. We will further discuss the possible mechanisms underlying metabolic dysfunction in OSA, which include several pathophysiological pathways triggered by the characteristic features of OSA, namely sleep disturbance and IH.

Clinical and epidemiological data

Association between obstructive sleep apnoea (OSA) and type 2 diabetes mellitus (T2DM)

OSA in diabetic cohorts
Given their shared relationship with obesity, a substantial burden of sleep disordered breathing in diabetic cohorts and a high prevalence of T2DM in OSA populations is perhaps to be expected. However, there is increasing evidence that the two disorders may have an independent link, one beyond a simple shared association with obesity. OSA is certainly highly prevalent in patients with T2DM. When 240 subjects attending British hospital- and community-based diabetes clinics underwent domiciliary overnight oximetry, with confirmatory cardio-respiratory sleep studies performed on those whose oximetry was suggestive of sleep disordered breathing, 23% were found to have OSA [defined as an oxygen desaturation index (ODI) >10/h] (3). An even greater prevalence of OSA was found among 305 obese American diabetics evaluated using home polysomnography (PSG). In this cohort, only 13.4% did not have sleep disordered breathing, with 22.6% classified as severe OSA (4). A key explanatory factor for the discrepancy between these two studies was the degree of obesity within their cohorts—subjects in the US-based Sleep-AHEAD study had a mean body mass index (BMI) of 36.1 kg/m^2, compared with 28.8 kg/m^2 in the English group.

Prevalence of T2DM in OSA cohorts
Diabetes is also over-represented in patients with OSA when compared with the general population (*Table 1*). The most useful data in this regard is derived from large community-based studies. In the Wisconsin sleep cohort study, physician-diagnosed T2DM was present in 1.5% of participants with an apnoea/hypopnea index (AHI) <5, but 7.8% with an AHI ≥15 (5). Similarly, 8.8% of men and 7.7% of women with an AHI <5 in the Sleep Heart Health Study had known T2DM, compared with 16.9% and 16.7% respectively of those with an AHI ≥30 (14). In a large Canadian sleep clinic cohort, 4.4% without OSA had an antecedent diagnosis of

diabetes, *vs.* 15.3% with severe OSA (6).

The increased prevalence of T2DM in patients with more severe OSA is not completely explained by traditional risk factors for metabolic disease. In a community-based analysis of 1,387 subjects of the Wisconsin Sleep Cohort, 23% of whom had an AHI ≥5, moderate-severe OSA was independently associated with prevalent physician-diagnosed T2DM, despite adjustment for age, gender, and body habitus [adjusted odds ratio (OR) =2.30; 95% CI, 1.28-4.11] (5). This relationship was still stronger in those with an AHI ≥30 (adjusted OR =3.48; 95% CI, 1.69-7.18). Among 2,149 patients referred to Canadian sleep clinics for diagnostic testing, severe OSA conferred an adjusted OR of 1.82 (95% CI, 1.07-3.10) for concomitant T2DM (6). In this study diabetes was defined according to self-report, physician diagnosis, medication usage, or from administrative database records. Perhaps surprisingly, this relationship appeared to be confined to sleepy subjects—those with an Epworth Sleepiness Score (ESS) score ≥10 had an adjusted OR of 2.59 (95% CI, 1.35-4.97) for prevalent diabetes, as compared with 1.16 (95% CI, 0.31-4.37) for those without subjective daytime sleepiness.

An important limitation of population-level studies of T2DM prevalence in sleep disordered breathing is their reliance on patient self-reporting, administrative databases or fasting plasma glucose measurement to establish a diagnosis of T2DM. The European Sleep Apnoea Cohort (ESADA) study is a multi-centre, multinational study involving over 15,000 patients attending sleep laboratories across Europe, Israel and Turkey. Participants in ESADA are screened at enrolment with glycosylated haemoglobin (HbA1c) measurement, which has recently been approved as a stand-alone diagnostic test for T2DM (15), and may identify a higher proportion of diabetics in sleep cohorts than reliance on fasting plasma glucose (16). In an analysis of nearly 7,000 subjects in the ESADA cohort, a diagnosis of severe sleep disordered breathing was associated with a markedly increased likelihood of prevalent T2DM, following adjustment for obesity, age, co-morbidities and medication use (adjusted OR =1.87; 95% CI, 1.45-2.42) (8).

Similarly, data from smaller, clinic-based studies using more robust methods to detect occult hyperglycaemia support an independent relationship between OSA and T2DM. In a French study of 595 men with suspected OSA undergoing PSG and subsequent oral glucose tolerance testing (OGTT), T2DM was found in 30.1% of subjects with an AHI >10, compared with 13.9% of non-apnoeic snorers (17). In a multivariate linear regression model, AHI

Table 1 Selected population studies population studies of associations between OSA and diabetes mellitus, glucose intolerance or insulin resistance

Authors	Participants	Study design	Outcome measures	Findings
OSA and type 2 diabetes mellitus				
Reichmuth *et al.* (5)	1,387 subjects community based	Observational	T2DM prevalence and incidence; T2DM defined by physician report or FPG	Increased prevalence of T2DM if AHI >15 (AOR 2.30); no increased incidence of T2DM with OSA after 4 years
Ronksley *et al.* (6)	2,149 subjects clinic based	Cross sectional	T2DM prevalence; T2DM defined by self-report and medication use	Independent association of severe OSA with T2DM (AOR 2.18); relationship confined to sleepy subjects
Botros *et al.* (7)	1,233 subjects clinic based	Observational	T2DM incidence	Increased incidence of T2DM with OSA (HR 1.43 per AHI quartile); CPAP use appeared to attenuate T2DM risk
Kent *et al.* (8)	6,616 subjects clinic based	Cross sectional	T2DM prevalence and control; T2DM defined by medication use, prior diagnosis or HbA1c ≥6.5%	Increased prevalence of T2DM with severe OSA (AOR 1.87); worse glycaemic control in diabetics with severe OSA
Kendzerska *et al.* (9)	8,678 subjects clinic based	Historical cohort	T2DM incidence; T2DM defined by related healthcare utilisation	Increased incidence of T2DM with severe OSA (HR 1.31)
OSA, insulin resistance and glucose intolerance				
Punjabi *et al.* (10)	2,656 subjects community based	Cross sectional	IGT measured by OGTT; IR estimated by HOMA-IR	Increased IGT with AHI >15 (AOR 1.46); elevated HOMA-IR with AHI >15 and increasing nocturnal hypoxaemia
Theorell-Haglow *et al.* (11)	400 female subjects community based	Cross sectional	Insulin sensitivity index derived from OGTT	Increasing AHI associated with reduced insulin sensitivity
Priou *et al.* (12)	1,599 subjects clinic based	Cross sectional	Pre-diabetes (HbA1c 6-6.49%)	Pre-diabetes more common with severe (AOR 2.02) or very severe OSA (AOR 2.96)
Kent *et al.* (13)	5,294 subjects clinic based	Cross sectional	Adjusted mean HbA1c levels by AHI quartile; pre-diabetes (HbA1c 6-6.49%)	Increasing HbA1c levels with increasing AHI; pre-diabetes more common in highest AHI quartile (AOR 2.12)

OSA, obstructive sleep apnoea; T2DM, type 2 diabetes mellitus; AHI, apnoea/hypopnea index; CPAP, continuous positive airway pressure; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance testing; HOMA-IR, homeostasis model assessment of insulin resistance.

was an independent negative predictor of insulin sensitivity. In a similar study, 129 Japanese patients with newly diagnosed OSA underwent OGTT; a post-load glucose in the diabetic range was found in 33.8% with an AHI ≥30 *vs.* 15% with an AHI <15 (18). In both of these studies, a large proportion of subjects had undiagnosed T2DM, emphasising the importance of rigorous screening in clinical and epidemiological studies.

Incidence of T2DM in OSA cohorts

There is some discordance in the published literature on how OSA may predict the subsequent development of T2DM. Studies evaluating snorers, performed largely without objective assessment of sleep-disordered breathing, are suggestive of a link. A questionnaire-based study of 2,504 Swedish men aged 30-69 assessed the relationship of self-reported snoring with development of self-reported T2DM over a 10-year period (19). The 2.4% of non-snorers developed T2DM over the study period, compared with 5.4% of snorers. In an analysis confined to obese participants, 13.5% of snorers and 8.6% of non-snorers reported incident T2DM. A complimentary study of 69,852 US

female nurses found regular snorers had an adjusted OR of 2.02 (95% CI, 1.71-2.40) for incident T2DM over a decade of follow-up compared with non-snorers, following adjustment for obesity, age and other risk factors for metabolic disease (20).

Few studies have longitudinally evaluated if objectively measured sleep disordered breathing constitutes an independent risk factor for T2DM. The 26 new cases of T2DM occurred among 978 participants in the Wisconsin sleep cohort over 4 years of follow-up, and in unadjusted analysis moderate-severe OSA was associated with an increased likelihood of incident diabetes mellitus (OR =4.06; 95% CI, 1.86-8.85) (5). This relationship was abolished following adjustment for age, gender, and body habitus (adjusted OR =1.62; 95% CI, 0.67-3.65; P=0.24), and no alteration in risk of T2DM was seen in those commenced on CPAP treatment. Conversely, in a study of 544 subjects referred for assessment of suspected sleep disordered breathing with a mean duration of follow-up of 2.7 years, T2DM occurred at a rate of 5.5 cases per 100 patient years in OSA patients, as opposed to 1.8 cases per 100 patient years in non-apnoeic snorers (7). This relationship survived adjustment for confounding variables [adjusted hazard ratio (HR) per AHI quartile =1.43; 95% CI, 1.10-1.86), and CPAP significantly attenuated the risk of incident T2DM (HR =0.53; 95% CI, 0.28-0.99).

Similarly, a recent historical cohort study, evaluating over 8,000 non-diabetic Canadian subjects attending a sleep laboratory, found that after a median follow-up period of 67 months, and following statistical adjustment for confounding factors, subjects with severe OSA were 30% more likely to develop clinically overt T2DM than those with an AHI <5 (9).

Glycaemic control in subjects with diabetes mellitus and obstructive sleep apnoea (OSA)

The presence of sleep disordered breathing may also contribute to poor diabetic control. In a study of 60 US patients recruited from a hospital diabetes clinic, 77% were found to have OSA (21). Increasing OSA severity was associated with increased HbA1c levels after adjustment for demographic, anthropometric and clinical variables, with an adjusted mean HbA1c level 3.69% higher among diabetics in the severe OSA group than in the non-apnoeic group. HbA1c also correlated significantly in multivariate linear regression analysis with AHI and other indices of sleep disordered breathing.

Among over 1,100 subjects with T2DM participating in the ESADA study, increasing OSA severity predicted increased likelihood of suboptimal glycaemic control (HbA1c >7%) (22). When compared with non-apnoeic diabetic patients, those with severe OSA had an adjusted OR of 2.02 (95% CI, 1.11-3.66) for poor diabetic control, following adjustment for confounding factors including prescription of diabetic medications (8). Similarly, adjusted mean HbA1c levels among diabetic patients increased with increasing OSA severity, from 6.76% (95% CI, 6.39-7.13) in those with no OSA to 7.48% (95% CI, 7.18-7.79) in those with severe OSA.

Treatment of obstructive sleep apnoea (OSA) and glycaemic control

The ability of CPAP therapy to make a meaningful impact upon diabetic control remains unclear (*Table 2*). Insulin sensitivity, as measured by hyperinsulinaemic euglycaemic clamp, improved significantly from 11.4±6.2 to 15.1±4.6 µmol/kg/min in 10 Australian diabetic subjects with significant OSA after 4 months CPAP (31). In another small study of 9 German diabetics, 3 months of CPAP improved insulin sensitivity, but had no significant impact upon HbA1c levels (32). A novel study of 20 diabetics with newly diagnosed OSA (AHI >15) evaluated nocturnal interstitial glucose measurements before and after 3 weeks of CPAP therapy, finding a significant decrease with CPAP [from (122.0±61.7) to (102.9±39.4) mg/dL; P=0.03] despite many of the participants gaining weight between the two studies (33). Unsurprisingly given the short duration of this study, no change was seen in HbA1c levels.

Conflicting results were produced by two of the more robust studies in this area. Babu *et al.* evaluated 25 obese diabetic subjects with OSA who were naïve to CPAP treatment (24). HbA1c levels and fasting interstitial glucose were measured before and after an average of 83 days of CPAP use. No significant change was observed in HbA1c levels in the overall cohort, but analyses restricted to subjects with a baseline HbA1c ≥7% or those with objective CPAP usage >4 h/night showed a reduction in HbA1c measurements. Furthermore, the improvement in glycaemic control correlated significantly with the number of nights of CPAP use (r=0.74; P=0.006). In the only randomised controlled trial specifically assessing the influence of CPAP therapy on diabetic control, 42 men with known T2DM and newly diagnosed OSA (ODI >10) were given therapeutic or sham CPAP for 3 months (23). As expected, those in the treatment group had improvements in daytime sleepiness and maintenance of wakefulness test results. However,

Table 2 Selected studies examining the effect of CPAP on diabetes, glucose intolerance and insulin resistance (IR)

Authors	Participants	Study design	Outcome measures	Findings
West *et al.* (23)	42 diabetic men, 4% ODI >10	RCT: CPAP *vs.* Sham (3 months)	Insulin resistance; HbA1c levels	No significant improvement with therapeutic CPAP
Babu *et al.* (24)	25 diabetics with OSA	Observational: median 83 days CPAP	Continuous glucose monitoring; HbA1c levels	Improved post-prandial glucose levels post-CPAP; improved HbA1c in subjects with baseline HbA1c >7%
Barcelo *et al.* (25)	44 diabetics with OSA	Observational: 3 months CPAP	Insulin levels; HOMA-IR	Reduced insulin resistance with CPAP in sleepy subjects only
Hoyos *et al.* (26)	65 non-diabetics, AHI >20	RCT: CPAP *vs.* Sham (12 weeks)	Insulin sensitivity; HOMA-IR	No significant improvement with therapeutic CPAP
Lam *et al.* (27)	61 men, FPG <6.1, AHI >15	RCT: CPAP *vs.* Sham (1 week)	Insulin sensitivity	Improved insulin sensitivity with CPAP; changes not maintained at 12-week follow-up, except in overweight subjects
Weinstock *et al.* (28)	50 subjects, AHI >15, impaired glucose tolerance	Crossover RCT: CPAP *vs.* Sham (8 weeks)	Resolution of IGT on OGTT; insulin sensitivity	No difference in likelihood of achieving normal OGTT; CPAP improved insulin sensitivity in subjects with AHI >30
Coughlin *et al.* (29)	34 non-diabetic subjects, mean RDI 40	Crossover RCT: CPAP *vs.* Sham (6 weeks)	HOMA-IR; resolution of metabolic syndrome	No significant difference in IR, strong trend in favour of CPAP; no difference in overall metabolic syndrome
Harsch *et al.* (30)	40 non-diabetic subjects, mean AHI 43	Observational: CPAP (3 months)	Insulin sensitivity	Improved insulin sensitivity after 2 days, maintained at 3 months; more significant improvement if BMI <30

CPAP, continuous positive airway pressure; ODI, oxygen desaturation index; RCT, randomised-controlled trial; OSA, obstructive sleep apnoea; HOMA-IR, homeostasis model assessment of insulin resistance; AHI, apnoea/hypopnea index; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance testing; RDI, respiratory disturbance index.

no improvements in HbA1c levels or measures of insulin sensitivity were observed. Overall CPAP compliance in the treatment arm was sub-optimal (average nightly use 3.6 h), but even with the exclusion of those who were particularly non-compliant, there was no benefit seen in glycaemic control with CPAP. Hence, it remains unproven if CPAP can act as an adjunct in diabetes control in diabetic subjects with sleep disordered breathing.

Association between obstructive sleep apnoea (OSA), insulin resistance (IR) and glucose intolerance

Insulin resistance (IR), glucose intolerance and obstructive sleep apnoea (OSA)

IR among non-diabetic subjects is associated with subsequent increased risk of T2DM and cardiovascular morbidity, and is an independent predictor of overall and cardiac mortality (34). Attempts to evaluate the relationship of OSA with IR are again complicated by the pivotal

role played by obesity in driving these disorders, but an expanding evidence base is supportive of the existence of an independent link.

Clinic-based and case control studies from North America, Asia and Europe have suggested that the presence and severity of OSA is independently associated with IR. Among 270 non-diabetic subjects attending a Hong Kong sleep clinic, 68.5% of whom had an AHI ≥5, homeostasis model assessment of insulin resistance (HOMA-IR) was higher in those with OSA and increased with increasing OSA severity (35). This relationship survived adjustment for confounding factors, and was observed in both obese and non-obese subjects. Punjabi *et al.* studied 150 middle-aged, overweight or mildly obese men, who were free of known cardiometabolic disease (36). An AHI ≥5 was associated with an increased risk of glucose intolerance (OR =2.15; 95% CI, 1.05-4.38) independently of the effect of obesity.

Data focusing on morbidly obese subjects and women have shown similar relationships. Severity of sleep

disordered breathing predicted an elevated HOMA-IR in 90 patients recruited from a bariatric clinic (37), while increasing OSA severity was associated with decreased insulin sensitivity in a cohort of 400 non-diabetic Swedish women (11). In the majority of these studies, measures of nocturnal hypoxaemia had the most robust relationship with impaired glycaemic health, suggesting a potential pathophysiological role of IH. Underscoring this, data from 394 participants in the Cleveland Family Study showed the overall burden of nocturnal hypoxaemia (expressed as the cumulative sleep time with SpO_2 <90%) to be the key predictor of impaired glucose tolerance (IGT) in OSA patients (38).

There are limited population-level data in this area (*Table 1*). In a community-based North American study of 2,656 subjects, 8.7% with a respiratory disturbance index (RDI) <5 had IGT on fasting plasma glucose measurement *vs.* 17.5% with an RDI ≥15, a relationship which persisted after statistical adjustment for confounding variables (adjusted OR =1.46; 95% CI, 1.09-1.97) (10). In an evaluation of the relationship of HbA1c levels with indices of OSA severity in 1,599 subjects without diabetes attending French sleep laboratories (12), multivariate regression analysis showed an association between increased HbA1c levels and OSA severity, most marked in patients with an AHI ≥50 (adjusted OR =2.96; 95% CI, 1.58-5.54). Most recently, a similar analysis of 5,294 non-diabetic participants in the pan-European ESADA study, showed a dose dependent relationship between glycaemic health and severity of sleep disordered breathing, with a significantly higher adjusted HbA1c level in highest AHI quartile (5.50%; 95% CI, 5.46-5.53%) than in the lowest (5.24%; 95% CI, 5.21-5.27%) (13).

Treatment of obstructive sleep apnoea (OSA) and insulin resistance (IR)

The effect of CPAP therapy on insulin sensitivity in non-diabetic subjects remains controversial, with uncontrolled studies suggesting some benefit, but randomised trials providing much more uncertain data (*Table 2*). Insulin sensitivity improved with two nights of nocturnal CPAP in 40 treatment-naïve, non-diabetic German subjects (30). This effect was maintained at 3 months, but appeared to be largely confined to non-obese patients. A Spanish study of 44 men with newly diagnosed OSA also found treatment with CPAP to be associated with reductions in HOMA-IR and insulin levels, but only in subjects with objective daytime sleepiness at baseline (25).

A randomised, double-blind, crossover trial enrolled 35 British patients free of diabetes, including subjects with known IR (29). Six weeks of CPAP lowered HOMA-IR to a greater degree than sham CPAP, but the difference did not reach statistical significance (Δ=–0.6; 95% CI, –1.3-0.1; P=0.08), whereas daytime sleepiness and blood pressure improved significantly with treatment. Meanwhile, a study of 61 Chinese subjects with an AHI ≥15 randomised to therapeutic vs. sham CPAP for 1 week found insulin sensitivity improved significantly in the treatment group (27). However, a longer term follow-up of the patients who received therapeutic CPAP found no significant change from baseline insulin sensitivity at 3 months, and the authors excluded all subjects with known IR, making its applicability to other OSA cohorts uncertain. Similarly, data from the United States and Australia suggest that CPAP may only have a beneficial effect on glucose tolerance in individuals with severe OSA (28), or on insulin sensitivity after prolonged utilisation (26), respectively.

A number of recent meta-analyses have assessed the effect of CPAP therapy on IR in non-diabetic patients with OSA. These analyses included both uncontrolled studies and randomised-controlled trials (RCTs), but overall did suggest some reduction in HOMA-IR with medium-term use of CPAP, at least amongst compliant patients (39,40). However, there is a clear need for large, well-designed RCTs in this area.

Obstructive sleep apnoea (OSA) and metabolic syndrome (MetS)

While a number of competing, more precise definitions exist (41,42), the MetS is broadly composed of coexistent IR, hypertension, abdominal obesity and dyslipidaemia, and is associated with increased cardiovascular mortality (43). MetS is highly prevalent in OSA cohorts (44), occurring in up to 87% of OSA patients in some series (45). Once again, the confounding effect of visceral adiposity has made it difficult to definitively identify an independent relationship between the two conditions.

There are only a limited number of well-conducted studies examining the effect of CPAP therapy on MetS as an entity. When 34 British subjects with moderate-severe OSA were commenced on 6 weeks of CPAP in a sham-controlled, cross-over fashion, daytime sleepiness and BP improved on CPAP, but the overall prevalence of MetS did not (29). Similarly, in a secondary analysis of a study involving 65 men randomised to therapeutic or sham CPAP for

12 weeks, no significant differences were observed between the two groups in regression or development of MetS (46). A number of uncontrolled studies have suggested that CPAP may be beneficial in this context (47), and CPAP certainly can improve individual component factors of MetS, particularly hypertension (48). However, there is a need for large, prospective RCTs in this field.

In summary, OSA is associated with increased likelihood of T2DM, IR and MetS, and diabetic subjects with severe OSA appear to be less likely to achieve adequate glycaemic control. However, it remains to be established if CPAP therapy can improve metabolic health in pre-diabetic or diabetic subjects.

Potential mechanisms of glucose metabolic dysregulation in obstructive sleep apnoea (OSA)

The pathophysiological mechanisms of alterations in glucose metabolism in OSA are incompletely understood. The process is likely multifactorial and our current concept involves sympathetic nervous system overactivity, systemic and adipose inflammation, oxidative stress and hormonal alterations among the most important pathways. Although the evidence for a causal link remains limited, the major characteristics of OSA, namely sleep fragmentation/ deprivation and IH, likely play pivotal roles as triggering factors of the pathophysiology.

Sleep fragmentation/deprivation and glucose metabolic dysfunction

Sleep fragmentation and reduction in total sleep time are characteristic features of OSA. Although this subject is poorly investigated directly in OSA cohorts, one could extrapolate data on sleep fragmentation and reduction in other settings. There is a stream of laboratory and epidemiological data showing an association of these features with T2DM and IR. Several cross-sectional and longitudinal studies, mainly using self-reported sleep duration, suggest that short sleep duration (generally defined as less than 6 h of sleep) is associated with increased risk of T2DM (49-54). In a recent meta-analysis, the relative risk (RR) for the development of T2DM with short sleep was estimated as 1.28 (55). In experimental studies, restricting sleep duration in healthy young subjects leads to alteration in glucose metabolism indicated by a decrease in insulin sensitivity, with subsequent reduced glucose tolerance (56-58).

Independently of reductions in sleep duration, sleep fragmentation with suppression of slow wave sleep also results in impaired insulin sensitivity without compensatory increase in insulin release, as demonstrated in a recent study on healthy volunteers (59). In addition, prospective population-based studies have shown an association between self-reported poor sleep quality and incident T2DM (53,60-62).

The mechanisms by which sleep deprivation and fragmentation contribute to alterations in glucose metabolism are incompletely understood. Sleep deprivation has been found to induce a pro-inflammatory state, with an increase in the release of interleukin (IL)-6 and tumour necrosis factor (TNF)-α and decreased adiponectin levels (63-66)—alterations which have been linked to IR (67). However, negative findings have also been reported, possibly due to considerable variability in baseline levels of these markers and normal circadian fluctuations (68-70). Gender differences have also been observed, and some studies have suggested that sleep restriction was associated with elevation in pro-inflammatory markers in men but not in women (66).

There is also compelling evidence for a role of sympathetic activation—which negatively affects insulin secretion and sensitivity (71,72)—in response to sleep deprivation and fragmentation, reflected in an increase in blood pressure, lower heart rate variability and decreased baroreflex sensitivity (58,73). Some studies also reported increased catecholamine concentration following sleep deprivation (56,57,69). Disturbances in the secretory profiles of the hormones cortisol and growth hormone (GH) may also contribute to the pathogenesis. Cortisol inhibits insulin secretion and elevations of cortisol in response to sleep deprivation and fragmentation have been detected in various studies (56,58,74-76). GH inhibits insulin activity, and usually peaks in the first half of the sleep period. Sleep restriction appears to be associated with a longer elevation of GH secretion during the night, and also a large secretory burst before sleep onset, leading to adverse effects on glucose regulation (77,78).

Several short-term laboratory studies support the assumption that short sleep alters the hormonal regulation of food intake by increasing levels of the appetite-stimulant hormone ghrelin, and by reducing levels of the suppressor leptin, leading to the subjective feeling of hunger with consequent increased caloric intake (79). Furthermore, there is evidence that daytime sleepiness and fatigue as a result of insufficient sleep may lead to reduction in physical activity and subsequent energy expenditure (80).

Collectively, sleep deprivation and fragmentation may contribute to metabolic dysfunction in OSA by various mechanisms. The direct relationships of these pathophysiological triggers in OSA populations, however, remain unknown, and there is a distinct lack of well-designed studies investigating this area.

Intermittent hypoxia (IH) and glucose metabolic dysfunction

IH, characterized by short repetitive cycles of desaturation followed by rapid reoxygenation, is the hallmark feature of OSA and is increasingly recognized as the major pathophysiological trigger for cardiovascular and metabolic disease processes associated with the disease (81). Cell culture and animal models have been developed, allowing the study of IH as a single component of the disease, precisely controlling the triggering events, both in severity and duration and thus, *in vitro* and animal studies have greatly contributed to our current knowledge. In lean and obese mice, chronic IH exposure contributes to decreased insulin sensitivity, an effect which is partially reversible after cessation of the stimulus (82-85). IH may affect glucose metabolism through systemic effects, including sympathetic excitation, inflammation or oxidative stress and through direct effects on adipose tissue, liver and pancreas.

Intermittent hypoxia (IH), sympathetic activation, systemic inflammation and oxidative stress

IH has been demonstrated to trigger multiple pathways potentially involved in the pathogenesis of cardiovascular and metabolic diseases (81). Sympathetic excitation in response to IH has been extensively studied in humans and rodents, and is widely believed to contribute to arterial systemic hypertension and other cardiovascular consequences (86). In addition, catecholamines are known to decrease insulin sensitivity and to reduce insulin-mediated glucose uptake (71,72). Several studies have demonstrated increased blood pressure and an associated rise in sympathetic activity in OSA patients versus matched controls, with improvement following short-term CPAP therapy (87-90). IH rodent models reproduce these changes, with blood pressure increases due to IH preventable by pharmacological and surgical blockage of the sympathetic nervous system (91,92). A recent study investigated the effect of adrenal medullectomy on glucose metabolism in mice treated with IH, and although surgery led to improved insulin secretion, IH-induced hyperglycaemia and IR remained unaffected, supporting the need for further

studies on this subject (93).

OSA-related IH has generally been associated with increased production of reactive oxygen species (ROS) and thus is thought to promote oxidative stress, which may contribute to alteration in glucose metabolism (94). Nonetheless, supporting data are sparse and results have been inconsistent. Jelic *et al.* harvested venous endothelial cells from OSA patients and matched controls, detecting higher levels of the oxidative stress marker nitrotyrosine in OSA subjects, with a significant fall following CPAP therapy (95). Studies in rats have demonstrated that IH leads to increased lipid peroxidation which correlated with left ventricular dysfunction (96). However, large studies have failed to show a beneficial effect of antioxidant therapy on cardiovascular diseases, and oxidative stress could potentially be a consequence rather than a cause of vascular inflammation and subsequent atherosclerosis (97).

Vascular and systemic inflammation are central in the pathogenesis of OSA-associated cardiometabolic processes and there is overwhelming support from human and animals studies of the key role of IH in driving inflammatory responses (98-100). IH activates the transcription factor nuclear factor-kappa B (NF-κB) (101-103). NF-κB is the master regulator of an inflammatory response, and numerous inflammatory, pro-atherogenic genes, such as TNF-α, IL-8 or intercellular adhesion molecule 1 (ICAM-1), are under its control. NF-κB activation has been demonstrated in cardiovascular tissue from mice treated with IH, and in cultured monocytes from OSA patients (101,103). Using a cell culture model of IH, our group demonstrated a preferential activation of NF-κB-dependent pro-inflammatory pathways by IH over adaptive, hypoxia-inducible factor 1 (HIF-1) mediated pathways, which is in contrast to sustained hypoxia where adaptive pathways predominate (102). Systemic and vascular levels of NF-κB-dependent pro-inflammatory mediators have been found to be increased in OSA patients *vs.* controls, potentially contributing to endothelial dysfunction, and CPAP therapy has a beneficial effect on these effects (100,104,105).

Effect of IH on adipose tissue

There is increasing evidence for an interaction between OSA and obesity in the development of cardiovascular and metabolic diseases. Obesity is strongly associated with OSA—the prevalence of OSA in obese subjects exceeds 30% and at least 60% of OSA patients are obese (106). Obesity exerts many of its cardiovascular and metabolic complications through the action of the white adipose tissue (WAT). WAT has been identified as a highly active endocrine

organ secreting multiple proteins—termed adipokines—which contribute to many pathophysiological processes (107). Metabolically dysfunctional adipose tissue is characterized by infiltration of macrophages and other immune cells including T-lymphocytes and mast cells. In addition, macrophages associated with obesity are polarized to a M1 or "classically activated" phenotype, and these M1 macrophages produce pro-inflammatory cytokines such as IL-6 and TNF-α, express inducible nitric oxide synthase (iNOS) and promote obesity-induced IR. In contrast, in lean subjects macrophages predominantly show an alternative, M2-pattern of expression with upregulation of anti-inflammatory factors such as IL-10 or adiponectin, and downregulation of pro-inflammatory cytokines, and thus are associated with tissue repair and resolution of inflammation (108).

There is emerging evidence that in obesity, where there is excess of adipose tissue, hypoxia is a key factor in modulating the pro-inflammatory response of WAT (109). As IH represents a stronger inflammatory stimulus than sustained hypoxia, this process may be potentiated by IH associated with OSA. Various studies have demonstrated that WAT in obese humans or rodents is more hypoxic than in lean controls (110,111). Given the technical difficulties in the direct measurement of rapid fluctuations in oxygen concentrations in tissue there are currently no data on the potential additive effect of IH in OSA subjects. Reinke *et al.* investigated the oxygen profile in response to IH in a mouse model and suggested that the oxygen fluctuations of IH are attenuated in adipose tissue (112). It is unknown, however, how this potentially relates to human adiposity and it is likely that there will be significant local differences within tissue depending on the relative distance to the circulatory system.

The master transcriptional regulator in response to hypoxia is HIF-1, which regulates transcription of numerous genes affecting various processes such as angiogenesis, metabolism, vascular tone and cell survival (113). It has recently been shown that HIF-1 activation occurs at the onset of obesity as a response to relative tissue hypoxia leading to adipose tissue inflammation and metabolic dysfunction (114); importantly genetic or pharmacological inhibition of HIF-1 proved protective in this setting. Meanwhile, hypoxia has previously been shown to inhibit insulin signalling and glucose transport in human and murine adipocytes in a HIF-1 dependent manner (115). Adipose HIF-1 activation in response to IH has also been recently demonstrated in ApoE-deficient lean mice, and this event was associated with accelerated atherosclerosis (116). Using the same

mouse strain, the hypoxia research group of the University of Grenoble, France, recently demonstrated increased macrophage infiltration of adipose tissue and increased expression of monocyte chemoattractant protein (MCP)-1, abnormalities which were associated with reduced insulin sensitivity and more advanced atherosclerotic lesions (117). However, in morbidly obese patients, IH does not appear to worsen macrophage accumulation in omental and subcutaneous fat depots (118), but there are no data outside the morbidly obese population. In support of a mechanistic link between IH and adipose tissue, Gharib *et al.* identified a profound effect of IH on gene expression in WAT mapping to numerous processes, including metabolism and oxidative stress responses (119). As a potential explanation, primary human adipocytes exposed to IH *in vitro* are significantly more sensitive to this pro-inflammatory stimulus than other primary cells, with the downstream consequence of increased activation of the NF-κB pathway and resultant expression of multiple inflammatory mediators known to play key roles in glucose metabolic dysfunction (120).

In summary, rapidly increasing evidence points to an additive effect of IH on adipose tissue inflammation, but further translational studies are urgently required to determine its detailed role.

Effect of IH on liver damage

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease characterized by the presence of ectopic fat in the liver, steatosis, which cannot be explained by alcohol consumption. It ranges from simple steatosis to non-alcoholic steatohepatits (NASH), that can progress to liver cirrhosis and is associated with the development of hepatocellular carcinoma (121). NAFLD is strongly associated with obesity, IR/T2DM and MetS (122). The relationship of NAFLD and IR is bi-directional, suggesting a self-perpetuating vicious cycle. Aron-Wisnewsky *et al.* systematically studied liver histology and overnight oximetry in morbidly obese patients undergoing bariatric surgery, and detected that the severity of IH—as indicated by the ODI—was associated with more severe liver injury, consistent with results of previous studies on OSA populations (118). Animal studies strongly support the detrimental role of IH on NAFLD progression, particularly evident in obese mice (82,123). The mechanisms underlying IH as "second hit" stimulus for NAFLD progression are largely unexplored and warrant further detailed studies. Furthermore, the role of CPAP therapy in potentially preventing NAFLD progression will need to be determined.
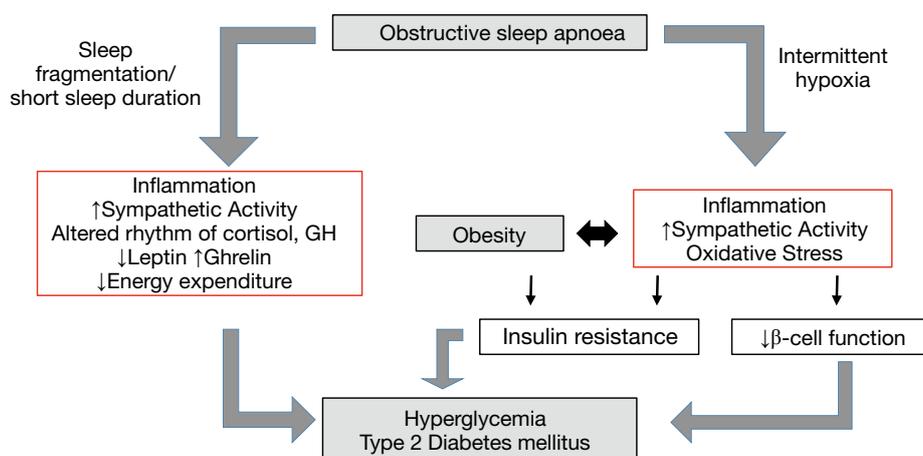
Figure 1 Potential mechanisms of alteration in glucose metabolism in obstructive sleep apnoea (OSA). GH, growth hormone.

Effect of IH on pancreatic B-cell function

B-cell dysfunction in response to IH is suspected based on the frequent observation that decreases in insulin sensitivity are often not matched by compensatory increase in insulin secretion. Louis *et al.* exposed a group of healthy volunteers to 8 h of IH or intermittent air in a randomized crossover design study, and despite worsening insulin sensitivity with IH, pancreatic insulin secretion was similar between both treatments (124). These results have subsequently been reproduced in mice and rats exposed to IH (83,84,125,126) and as a potential explanation, Polak *et al.* detected increased protein levels of the oxidative stress marker thiobarbituric acid reactive substances in pancreas isolated from mice treated with IH *vs.* intermittent air (84). The detailed mechanisms including the role of oxidative stress in this process, nonetheless, warrant further detailed investigation.

Conclusions and future directions

Evidence for an association of OSA and glucose metabolic dysfunction is rapidly growing. However, much of this evidence is derived from cross-sectional analyses and there is a clear need for large, well-designed, prospective studies. The lack of benefit with CPAP therapy in numerous studies demands a greater insight into the interaction of OSA and other contributing factors, in particular obesity, in the pathogenesis of alterations in glucose metabolism. IH and sleep deprivation/fragmentation likely play pivotal roles in the pathogenesis of glucose metabolic dysfunction in OSA, potentially contributing to multiple pathways likely synergistically with obesity (*Figure 1*). To further define

the mechanisms underlying these processes, clinical and translational studies are urgently required.

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Footnote

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New developments in the use of positive airway pressure for obstructive sleep apnea

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Abstract: Obstructive sleep apnea (OSA) is a disorder which afflicts a large number of individuals around the world. OSA causes sleepiness and is a major cardiovascular risk factor. Since its inception in the early 1980's, continuous positive airway pressure (CPAP) has emerged as the major treatment of OSA, and it has been shown to improve sleepiness, hypertension, and a number of cardiovascular indices. Despite its successes, adherence with treatment remains a major limitation. Herein we will review the evidence behind the use of positive airway pressure (PAP) therapy, its various modes, and the methods employed to improve adherence. We will also discuss the future of PAP therapy in OSA and personalization of care.

Keywords: Obstructive sleep apnea (OSA); positive airway pressure (PAP); treatment adherence

Introduction

Obstructive sleep apnea (OSA) is a disorder characterized by intermittent reduction of breathing due to complete or partial occlusion of the upper airway during sleep (1). The prevalence of OSA has been rising over the last several decades in part due to the obesity pandemic. It is estimated that 13% of men and 6% of women have moderate to severe OSA defined as an apnea-hypopnea index (AHI) ≥15/hour (2).

First developed in the early 1980's (3), continuous positive airway pressure (CPAP) has become established as the treatment of choice for OSA (4). CPAP consists of a mask through which airflow is delivered by a blower (*Figure 1*). This servo-controlled fixed pressure is designed to overcome the tissue forces tending to collapse the upper airway thereby stenting the airway open. Because of this concept, CPAP is commonly referred to as a 'pneumatic splint' (*Figure 2*) (3).

CPAP as a treatment of OSA symptoms

A large literature exists demonstrating the efficacy of CPAP in treating OSA symptoms. Snoring, a very common presenting complaint, represents turbulent airflow caused by upper airway narrowing (6). Snoring can be bothersome to the bed partner adversely impacting their sleep quality and can lead to a loss of intimacy when it causes the patient and partner to sleep apart. In addition, vibration of the carotid arteries due to snoring may theoretically increase stroke risk independent of airway obstruction or hypoxemia (7). By preventing airway collapse/vibration, CPAP eliminates snoring (8). As a result, CPAP has been shown to improve sleep quality in the bed partner (9). Similarly, evidence suggests CPAP can reduce other nocturnal symptoms such as gasping or choking, nocturnal awakenings, and nocturia (10).

Another cardinal symptom of OSA is excessive daytime sleepiness (11,12). Randomized controlled trials have
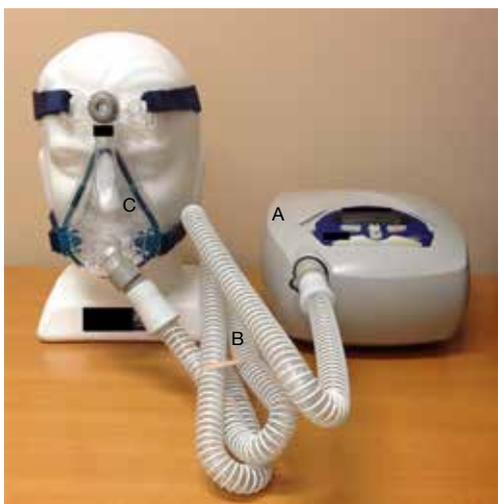
Figure 1 Representative photo of CPAP in place on a mannequin. (A) CPAP blower unit which creates pressure gradient; (B) CPAP tubing which transmits pressure to the mask; (C) CPAP mask which is applied firmly to the patient and provides positive pressure to the upper airway. CPAP, continuous positive airway pressure.

consistently demonstrated that CPAP improves sleepiness as assessed both objectively and subjectively (13). Compared to sham CPAP, one large meta-analysis found CPAP reduces sleepiness on the Epworth Sleepiness Scale (ESS) by an average of 4 points (13). Studies suggest that even in mild OSA associated with sleepiness, CPAP can improve symptoms (14). A major consequence of excessive daytime sleepiness is motor vehicle collisions. Observational studies suggest that CPAP therapy improves performance on driving simulator tasks (15), and CPAP therapy is associated with a decline in motor vehicle accident rate (16).

Another symptom commonly attributed to OSA is depressed mood. Results of clinical trials assessing the impact of CPAP on mood have been mixed with both positive and negative results. A limitation of the literature has been the lack of studies focusing on OSA patients with depressed mood at baseline (13). OSA may also affect cognitive function; however, the Apnea Positive Pressure Long-term Efficacy Study (APPLES), the largest randomized trial in OSA done to date, did not demonstrate

0 cmH$_2$O   15 cmH$_2$O
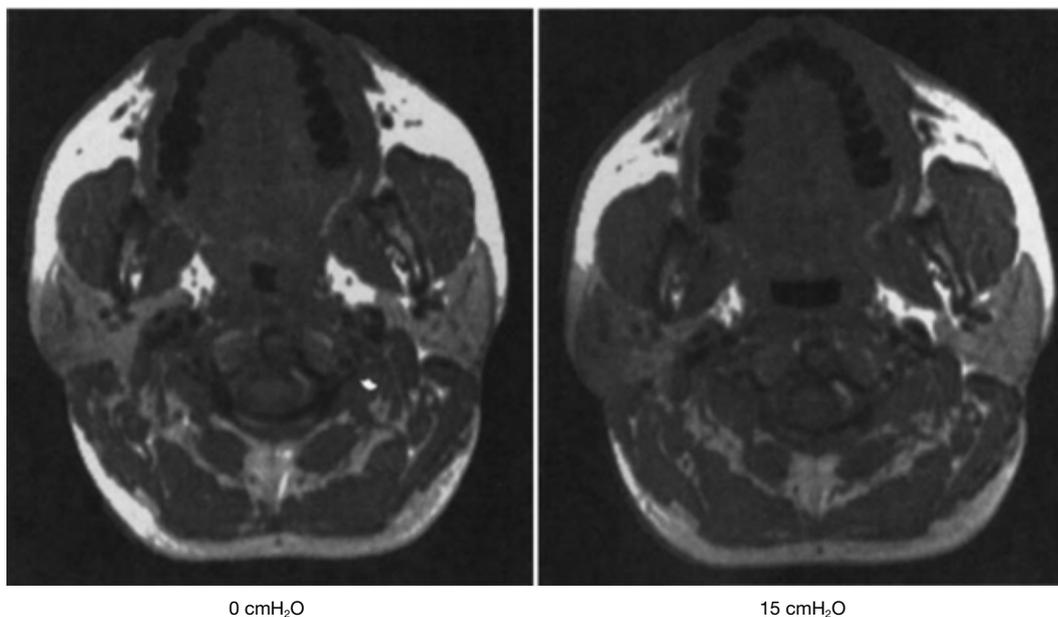
Figure 2 Effect of positive airway pressure on cross sectional upper airway anatomy. (Schwab *et al.*, AJRCCM, 1996; reprinted with permission of the American Thoracic Society. Copyright© 2014 American Thoracic Society) (5).

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any sustained improvements in cognitive function despite detailed testing in multiple domains. Criticisms of this trial were the large number of dropouts and that the enrolled population had normal cognitive function at baseline (17). Whether CPAP improves function or prevents decline in those with cognitive impairment at baseline is unclear, although a small randomized trial in patients with Alzheimer's dementia suggested potential benefits (18,19).

Furthermore, CPAP has consistently been shown to produce improvements in OSA-specific quality of life measures (20,21). However, when assessed using global quality of life measures such as the 36-Item Short Form Health Survey (SF-36), a meta-analysis of eight studies suggests the impact of CPAP on global quality of life is relatively small, with the greatest benefit in the domains of physical function, general health, and vitality (13).

CPAP to improve long term outcomes

In addition to symptoms, OSA is associated with a host of cardiovascular risk factors and adverse outcomes including hypertension, stroke, and heart failure, leading many clinicians to recommend treatment of OSA even in asymptomatic patients. Among the OSA treatments available, CPAP has the strongest evidence for a beneficial cardiovascular effect (22-30).

OSA has been well established as an independent risk factor for hypertension (31,32). Randomized trials show a small but consistent effect of CPAP therapy in lowering blood pressure. Based on a large meta-analysis, CPAP lowers systolic blood pressure (SBP) by 2.5 mmHg and diastolic blood pressure (DBP) by 1.8 mmHg (33). Of note, increased CPAP adherence predicts greater blood pressure reductions. In one trial, SBP and DBP fell by <1.5 mmHg in those using CPAP ≤5.65 hours per night, but fell by 3.7 and 5.6 mmHg respectively in those with >5.65 hours of usage (22). Some studies have found minimal to no effectiveness of CPAP therapy on blood pressure and other vascular measures in non-sleepy adults (22,34-36). However, a meta-analysis using individual patient data from these four studies demonstrated improvement in DBP (−1.4 mmHg) in those using therapy for >4 hours/night. These data would suggest that the lack of effect may be secondary to non-adherence, but biological variations in susceptibility to OSA consequences may also be important (37). It has also been postulated that the blood pressure response to CPAP may be greatest in those with resistant hypertension as a

small trial reported 6.5±3.3 and 4.5±1.9 mmHg drops in daytime SBP and DBP respectively (38). However, a larger trial found the 24-hour mean arterial pressure reduction was only 3.1 mmHg, similar to the magnitude in other hypertension studies (39).

In addition to hypertension, OSA is also independently associated with other components of the metabolic syndrome such as impaired glucose tolerance (40) and dyslipidemia (41,42). In clinical trials, CPAP has not been shown to improve fasting glucose in normoglycemic patients but has improved measures of insulin resistance on challenge testing (43-45). In a trial of patients with impaired glucose tolerance at baseline and moderate to severe OSA, CPAP did not improve glucose tolerance overall but there was evidence of improvements in post-hoc exploratory analyses among those with severe OSA at baseline (46). Finally, a small trial among patients with type 2 diabetes showed no improvement in glucose control with CPAP therapy (47). Regarding lipid levels, trials have been consistently negative in demonstrating an improvement in fasting lipid levels with CPAP (43,48,49). However, a small trial suggested 24-hour levels of triglycerides improve with CPAP therapy due to improvements in post-prandial levels (50). Several trials have assessed the impact of CPAP on visceral fat. Overall, these studies have not shown an impact of CPAP on visceral fat mass (45,51,52). However, there does appear to be an effect of CPAP on weight such that CPAP therapy is associated with a small but consistent increase in weight (53-55). Some have suggested this finding is due to reduced work of breathing at night though others have argued this observation represents an increase in muscle mass due to improvements in growth hormone function (56). Another possibility is that CPAP restores normal social activities which often involve caloric intake (e.g., dinner with spouse, beers with friends) leading to weight gain.

Data from the Sleep Heart Health Study have demonstrated between OSA and incident cardiovascular disease including stroke, coronary artery disease, and heart failure (25,29). The relationship of CPAP to stroke prevention remains unclear, but among stroke survivors with OSA, CPAP improves neurologic and motor recovery after stroke, delays appearance of cardiovascular events, and may prevent cardiovascular events (57,58). In observational studies, CPAP users experience a lower incidence of fatal and non-fatal coronary events compared to non-users (23,27). A large randomized trial conducted by the Spanish Sleep and Breathing Network did not
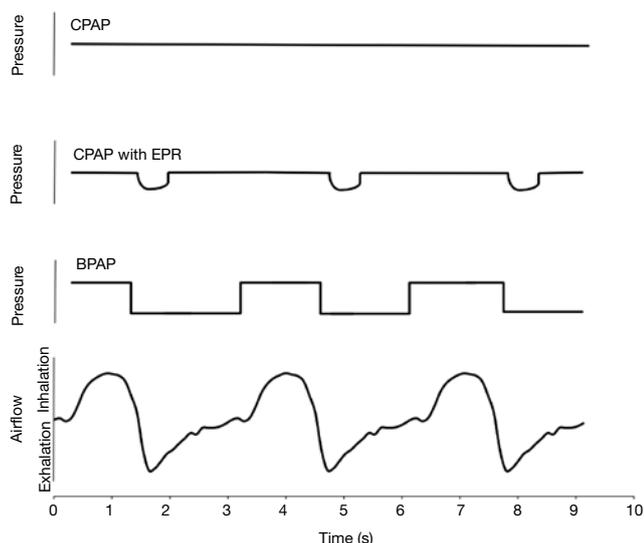
Figure 3 Representative airflow tracing with pressure time tracings of CPAP, CPAP with expiratory pressure relief (EPR), and bi-level positive airway pressure (BPAP). EPR decreases applied pressure level during exhalation in a flow-dependent manner. BPAP provides a fixed inspiratory pressure and a fixed expiratory pressure. CPAP, continuous positive airway pressure.

demonstrate a reduction in the combined endpoint of incident hypertension and cardiovascular events in people with asymptomatic OSA, although this trial may have been underpowered (22). Several larger randomized trials are currently underway to address more definitively the impact of CPAP on cardiovascular event risk. These include the Sleep Apnea Cardiovascular Endpoints (SAVE) study, the Randomized Intervention with CPAP Treatment in Coronary Artery Disease and Sleep Apnea (RICCADSA) trial, and the Continuous Positive Airway Pressure in Patients with Acute Coronary Syndrome and Obstructive Sleep Apnea (ISAACC) trial (59,60).

In short term trials, CPAP has been shown to improve left ventricular ejection fraction in patients with OSA and systolic heart failure (61,62). In patients with OSA and heart failure with preserved ejection fraction, CPAP use improves diastolic function (63). In addition, observational studies suggest CPAP use in OSA and heart failure is associated with a lower risk of death and hospitalization (64,65). In terms of pulmonary hypertension, a small randomized crossover trial found CPAP led to a 4.9 mmHg reduction in pulmonary artery systolic pressure (66). A number of studies have also evaluated the role of CPAP in the prevention of

atrial fibrillation. Observational studies have demonstrated that patients with treated OSA have approximately half the rate of recurrent atrial fibrillation following ablation compared to those with untreated OSA (67-69). However, because these results may be due to fundamental differences predicting adherence ("healthy user effect"), randomized studies in this population are needed.

Observational studies suggest that people with OSA also may have higher rates of incident cancer and cancer-related mortality (70,71). Clinical outcome data suggest individuals who use CPAP may have lower mortality compared to those who are untreated (72,73).

Interventions to improve adherence

Although CPAP is established as a highly efficacious treatment for OSA, its effectiveness has been limited by poor adherence. Users often experience nasal discomfort, congestion, mask leak and claustrophobia which lead to variable levels of long term compliance ranging from 46% to 85%, depending on how compliance is defined (74). Although adherence has been arbitrarily defined as usage for >4 hours/night more than 70% of nights for the purposes of insurance reimbursement in the United States, there does not appear to be a clear threshold above which adverse effects of OSA reverse (75). In contrast, there is a fairly linear dose response relationship such that the greater the CPAP usage, the greater the improvement in sleepiness, quality of life, or blood pressure outcomes (22,27). As a result, there has been much research on methods to optimize CPAP adherence.

Device manufacturers have developed advances in PAP technology as one means to improve acceptance and adherence. Advanced PAP features include auto-titrating PAP (APAP), bi-level PAP (BPAP), and expiratory pressure relief (EPR). A goal for all of these interventions has been to lower the pressure delivered to the airway due to the concern that higher pressures lead to reduced patient adherence (*Figure 3*). However, most observational data have not identified PAP level as a predictor of adherence (76,77).

Bi-level positive airway pressure (BPAP)

BPAP was the first device developed to try to lower mean airway pressures.

Instead of applying a fixed pressure throughout the respiratory cycle, BPAP applies a lower expiratory

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positive airway pressure (EPAP) during exhalation and a higher inspiratory positive airway pressure (IPAP) during inhalation (78,79). By maintaining IPAP above P_{crit}, the EPAP may be reduced without airway collapse. This approach can lower mean airway pressure particularly during exhalation when the patient has to breathe out against the delivered pressure. In the only large comparative trial evaluating BPAP with CPAP in PAP-naïve OSA (n=83), nightly usage between the two groups was similar as were the range and number of complaints. In fact, BPAP users with a large (greater than 6 cmH$_2$O) IPAP-EPAP difference had significantly lower compliance than CPAP users (80). Nevertheless, BPAP may be helpful in the subset of patients who complain of pressure intolerance. In addition, because BPAP machines can generate pressures above the maximal CPAP level of 20 cmH$_2$O, BPAP can be useful in the most severe OSA patients. Finally, use of a high IPAP to EPAP difference can be used to increase the tidal volume and so provide ventilatory support in people with hypoventilation syndromes (80). Retrospective data from a population of US Military Veterans indicate that BPAP is used more often in individuals with high BMI, CHF, COPD, hypercapnia, and severe hypoxia (81).

Auto-titrating devices

Instead of operating at a set pressure, APAP monitors a patient's respiratory activity in order to provide the lowest level of PAP necessary to eliminate respiratory disturbances (82). Algorithms are designed to increase pressure when events are noted and to decrease pressure slowly if events have not occurred for a period of time. Because the minimum PAP level necessary to eliminate airway obstruction varies over the night by position, sleep stage, and other factors, APAP has the capacity to provide the lowest pressure necessary at each time and therefore lower the overall mean pressure across the night (83). Compared to CPAP, APAP demonstrates a small but statistically significant superiority in adherence (+11 minutes) as well as in reduction of sleepiness (−0.5 points in ESS) (84,85). In contrast, evidence suggests that fixed CPAP level may be superior to APAP in blood pressure reduction and other cardiometabolic outcomes (85). For now, we regard fixed CPAP as the treatment of choice based on available data.

Another use of APAP has been to determine CPAP level requirements in the home rather than a sleep laboratory. Typically, the patient is provided an APAP device for 5-7 nights and the device is then interrogated to identify the 90th or 95th percentile pressure required which is inferred to be their fixed CPAP requirement. Such a protocol has been demonstrated to identify CPAP requirements effectively. A diagnostic protocol combining home sleep testing followed by APAP titration has been shown to reduce costs of OSA diagnosis and treatment substantially, lower time to treatment, and improve CPAP adherence as compared to the traditional strategy of in-laboratory polysomnography and CPAP titration (86).

It is important to note that each APAP manufacturer has their own proprietary algorithm for determining pressure changes and the optimal algorithm is unclear. Auto-titrating algorithms have also been developed for BPAP and a small study suggests auto-titrating BPAP may be helpful as a rescue therapy for occasional patients failing CPAP, although in aggregate the trial was negative (87).

Expiratory pressure relief (EPR)

Another strategy to improve compliance has been the use of EPR. Similar to BPAP, machines programmed to perform pressure relief provide less airway pressure during early exhalation compared to standard PAP at the same set pressure. Unlike BPAP, EPR varies with each breath according to the expiratory flow rate—a higher expiratory flow rate from the patient yields a greater EPR resulting in a greater pressure drop in early exhalation as opposed to late exhalation. An initial non-randomized evaluation found an impressive improvement in CPAP adherence (~1.5 hours/night) with EPR (88). However, five randomized trials have been subsequently performed and a meta-analysis of these trials did not identify an improvement in adherence with EPR (89). As with APAP, the EPR algorithm varies by manufacturer and several manufacturers provide advances beyond pressure relief in expiration alone including the addition of inspiratory pressure relief in late inspiration for patients on BPAP. A small trial comparing APAP alone, APAP with EPR, and APAP with both inspiratory and EPR found the highest level of adherence among those randomized to APAP with EPR (90).

Adaptive servo-ventilation (ASV)

A further advancement in PAP technology has been the development of ASV as a mode of therapy. In ASV, BPAP is delivered but the level of ventilatory support (IPAP-EPAP) provided varies over time. The goal of ASV is to eliminate obstructive events with the EPAP and to hold
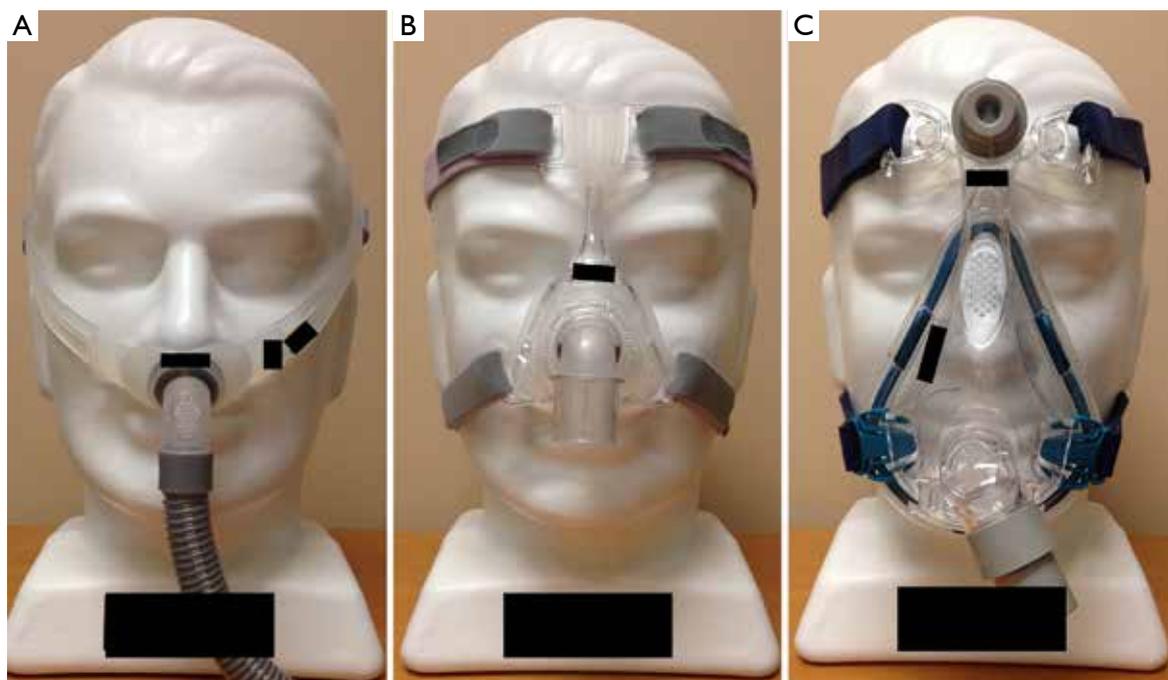
Figure 4 Representative masks. (A) Nasal pillows mask; (B) nasal mask; (C) oronasal mask.

ventilation at a fixed level by providing ventilatory support in inverse proportion to the patient's own ventilation. As a result, periodic breathing (in the timeframe over which the ASV algorithm's assessment of ventilation is being made) will be dampened out (91). ASV has shown high efficacy in resolving Cheyne-Stokes breathing associated with congestive heart failure alone or associated with OSA (92,93). However, a preliminary analysis of the Treatment of Predominant Central Sleep Apnoea by Adaptive Servo Ventilation in Patients with Heart Failure (SERVE-HF) phase III randomized trial, found an increased risk of cardiovascular mortality in those randomized to ASV (94,95). A more complete analysis of the results is pending to understand the cause of this unexpected finding. In the meantime, another randomized trial of ASV, the Effect of Adaptive Servo Ventilation (ASV) on Survival and Hospital Admissions in Heart Failure (ADVENT-HF), which includes those with OSA and those with milder levels of heart failure is ongoing (96). Some have advocated the use of ASV in the treatment of CPAP-emergent central apneas as well; however, a randomized trial of CPAP *vs.* ASV in CPAP-emergent central apneas found no benefit of ASV in terms of PAP adherence or symptoms. Thus, there is currently no strong rationale for the use of ASV in this setting (97).

CPAP modifications—humidification and mask choice

Early studies reported nasal dryness was noted in 44-65% of patients treated with nasal CPAP suggesting humidification may improve tolerance (98,99). Three randomized controlled trials have been performed evaluating the effect of adding heated humidification to the CPAP circuit. The first trial demonstrated a small improvement in adherence (100), but two larger subsequent trials found no benefit. Sleepiness has not been significantly affected by humidification (100-102). Nevertheless, given the possibility that heated humidification may improve compliance and satisfaction, it is now standard on all PAP machines available today, and can typically be turned off based on patient preference.

Given that as many as 50% of patients have at least one complaint regarding their mask interface (103), improving mask fit has been another target for increasing CPAP adherence. There are a wide range of mask types available today including nasal masks, oronasal masks, nasal pillow masks, hybrid oral masks with nasal pillows, and oral only masks (*Figure 4*). One small randomized trial found CPAP use was 1 hour per night greater with a nasal mask versus an oronasal mask (104). However, a subsequent meta-analysis found no consistent difference between mask types (105). Nevertheless, a large observational study found oronasal mask

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use was an independent predictor of non-adherence (76). Of note, the oronasal mask was typically prescribed as rescue therapy so these results may be confounded. Nevertheless, data demonstrating that pressure requirements may be higher with an oronasal mask suggest a nasal mask may be preferred as first choice (106,107). However, given the wide range of facial structures and nasal pathology, it is important to tailor mask choice to patient preferences.

Adjuvant hypnotic use

Because CPAP use may initially produce discomfort from the mask as well as anxiety, treatment of acute insomnia with short term use of a hypnotic drug has been advocated by some to reduce sleep latency and improve sleep continuity during the initiation of PAP therapy. A controlled clinical trial randomized OSA patients to 14 days of eszopiclone or placebo upon initiation of CPAP therapy. After 6 months, adherence was significantly better in the eszopiclone group by approximately 1 hour/night (108). In contrast, a similarly conducted trial using zolpidem found no benefit of hypnotic use (109). Another trial evaluating the effect of one night of zaleplon *vs.* placebo prior to a split night diagnostic polysomnogram/CPAP titration also found no difference in CPAP adherence 1 month later (110).

Education

Because reduced understanding of disease and treatment has been associated with lower levels of adherence in many chronic diseases, educational interventions to improve patient understanding of OSA and the benefits of CPAP have been developed. Several trials have been conducted to test the effect of intensive education regarding OSA and CPAP on patient compliance. Interventions that have been assessed include intensive education by practitioners and homecare providers (111) as well as use of standardized audiovisual presentations and demonstrations (112,113). To date, none of the educational interventions has consistently improved CPAP compliance. Many of these trials have been criticized in that the level of education provided to the control arm exceeds what is typically provided in routine care. As a result, most experts still recommend a basic level of education to all patients initiating CPAP therapy (114,115).

Motivational interviewing and cognitive behavioral therapy (CBT)

Psychosocial interventions with motivational interviewing and CBT have shown promise in augmenting CPAP compliance. Motivational interviewing is a type of psychosocial intervention in which a therapist elicits and targets an individual's behavior by assessing the subject's readiness to change, perceived importance of the change, and confidence in their ability to change. This motivational style has proven useful in smoking cessation (116) and the management of other chronic diseases (117). A three-arm randomized controlled trial comparing standard care, education, and motivational interviewing in CPAP naïve individuals demonstrated a trend towards increased adherence with the use of two 45-minute motivational interviewing sessions compared to standard care (118). A subsequent larger trial, however, did not identify a benefit at 12 months (119). Another trial using an intervention which involved three 30-minute motivational interviewing sessions over the first month of therapy demonstrated approximately 1.5 hours/night improvement in CPAP use at 3 months; however, no differences remained at 1 year (120). More recently, a Chinese trial randomized patients to either standard care or a pathway of interventions including educational and motivational visits and phone calls over 3 months tailored to their readiness to accept CPAP. The intervention group had greater CPAP adherence at 1 and 3 months (121). A less intensive strategy involving just one 20-minute interview and a 10-minute follow-up call using motivational interviewing was also successful in improving CPAP usage (122).

CBT, a psychosocial intervention aimed at correcting irrational and incorrect beliefs in order to alter behavior, has also been used to augment CPAP adherence. A randomized controlled trial evaluated the impact of two 1-hour group sessions (ten participants per group) compared to standard care and found those randomized to CBT used CPAP 2.9 hours longer than controls (123). These results indicate that behavioral therapy can have a large impact on CPAP adherence. However, subsequent research using a lower dose of CBT has not demonstrated a significant benefit, calling the feasibility of CBT as routine care into question (124).

Peer and spousal support

In addition to support from clinicians, the effect of peer

support in improving CPAP adherence has also been studied. In a cohort of military veterans, "peer buddies" (fellow OSA patients with good CPAP compliance) met with newly diagnosed patients twice to provide support. This intervention led to improved compliance compared to control (125).

A major source of support for patients can also be their bed-partners. In observational studies, the presence of a bed-partner is associated with greater CPAP adherence (126). The presence of a bed-partner can also have negative consequences. Concerns about sexuality and bed-partner disturbance can adversely affect CPAP use (127,128), and perception of spousal "pressure" to use CPAP is associated with worse adherence (129,130). Nevertheless, in severe OSA, perceived spousal support correlates with greater CPAP use (130), and a perceived collaborative relationship with one's spouse also predicts better adherence (131). Thus far, no studies have evaluated the impact of interventions aimed at improving bed-partner support.

Telemedicine

With the advent of the digital age, the application of telecommunication devices to ease communication between patient and provider has been applied to a number of chronic diseases (132-135). Unsurprisingly, a number of studies have been performed to evaluate the effect of telemedicine interventions on CPAP adherence. Using data wirelessly transmitted by patient's CPAP units, investigators in one study monitored compliance on a daily basis. If a patient was found to have excessive mask leak, high AHI, or low duration of use, a research assistant would call the patient and troubleshoot problems. After 3 months, the group assigned to the telemedicine intervention had 1.88 hours/night greater CPAP use (136). A similarly designed randomized trial evaluated the effect of remote compliance surveillance by study staff in addition to a web-based service in which the patient could observe their own usage data. Patients randomized to the telemedicine arm had greater adherence at 2 months (137). A limitation of both studies is the cost in time and effort for an individual to survey the compliance data regularly for a large sample of patients. A lower cost alternative has also been studied. In a veteran's population, patients were randomized to either usual care or a series of weekly automated telephone calls in which patients were able to report side effects of CPAP use and obtain pre-recorded advice. In addition, these reports were relayed to providers. At 1 year, patients

in the intervention group demonstrated significantly higher adherence rates. Of note, however, mean usage was poor in both groups (138).

Alternative and adjunctive therapies to PAP

Because CPAP cannot be tolerated by all patients, there is a role for other treatments either as alternative therapies or as adjunctive treatments to reduce PAP requirements.

Weight loss

Given that obesity is one of the strongest risk factors for OSA, weight loss clearly improves OSA severity (139,140). Randomized trials in mild OSA suggest weight loss programs can resolve OSA but even in more severe disease, weight loss reduces OSA severity and improves symptoms (141,142). Furthermore, exercise itself may improve OSA even in the absence of weight loss (143). Bariatric surgery has increasingly been advocated in the treatment of OSA. A recent trial comparing gastric banding with intensive medical weight loss as adjunctive treatments to CPAP, found at 2 years that the AHI had reduced by 31.6% in the surgical arm although this change was not significantly different from the medical arm. However, few patients in either arm were able to stop CPAP (144). Another trial assessing the impact of medical weight loss as adjunctive therapy to CPAP found weight loss tended to have a more beneficial impact on cardiovascular markers than CPAP and the greatest improvements were in those receiving both CPAP and weight loss (43). The importance of combining weight loss therapy with CPAP is further emphasized by emerging data demonstrating CPAP treatment leads to weight gain (17).

Sleep position

Due to the effects of gravity on upper airway anatomy, OSA severity is often worse in the supine position, and a substantial number of patients only have airway collapse while supine (145,146). In these patients, positional therapies can improve sleep apnea as monotherapy although long term compliance—even when assistive devices are employed—is only about 25% (147). In theory, combining CPAP with positional therapy may allow for the use of lower CPAP levels. A post-hoc analysis found the greatest benefit of APAP *vs.* CPAP was in those patients with highly positional disease, where presumably the APAP

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could use substantially lower pressures when patients were non-supine (148).

Mandibular advancement devices (MADs)

MADs have become firmly rooted as second line therapy in the treatment of OSA. Randomized trials have demonstrated the efficacy of MADs to reduce sleepiness and blood pressure in OSA (149,150). While CPAP improves the AHI to a greater extent than MAD therapy (149,151-153), patient satisfaction may be greater with MADs (154). A recent comparative effectiveness trial found no difference between CPAP and MAD in treating sleepiness due to greater improvement of OSA with CPAP but greater adherence with MAD (155). Of note, neither treatment improved blood pressure in this study. One small trial has assessed the impact of adding MAD to CPAP. In patients previously intolerant to CPAP, MAD reduced the applied pressure required, frequency of residual apneas, and residual sleepiness (156).

Surgery

The most commonly performed surgery to treat OSA is the uvulopalatopharyngoplasty (UPPP) which involves a tonsillectomy, excision of the uvula and posterior palate and trimming of the posterior pillars. This procedure lowers the AHI below 10/h in only 33% of patients (157). Furthermore, UPPP may compromise future CPAP use, as patients with UPPP are more likely to be non-compliant, have increased air leak, and lower pressure tolerance than matched controls (158). Maxillo-mandibular advancement (MMA) is a procedure which advances the anterior pharyngeal palate and enlarges the mandible. In uncontrolled studies, MMA is highly efficacious, resulting in a mean residual AHI of 7.7/hour (159). However, given the morbidity of the surgery, MMA is infrequently utilized (160). Tracheostomy cures OSA by bypassing the upper airway completely but is also infrequently utilized due to the high associated morbidity including recurrent pneumonia, stomal complications, and psychological trauma (161).

Oral pressure therapy (OPT)

OPT is a novel treatment option which involves the application of negative pressure in the mouth to pull the tongue anteriorly. A small, unblinded trial demonstrated a statistically significant reduction in AHI and an improvement

in subjective sleepiness with OPT. Compliance with the device, 6.0±1.4 hour, was higher than commonly seen with PAP (162). More definitive studies are needed to understand the role of OPT in OSA management.

Expiratory positive airway pressure (EPAP)

The application of EPAP at the nose through the use of one-way valves has also been developed as an alternative to CPAP. The valves freely allow inspiration, but inhibit expiration until a sufficient amount of expiratory pressure is generated. An initial randomized trial demonstrated improvements in AHI and sleepiness with EPAP therapy (163). However, a subsequent larger trial in prior CPAP users found a high failure rate with EPAP, not significantly different from placebo (164).

Hypoglossal nerve stimulation (HGNS)

Given the role of the upper airway dilator muscles in maintaining airway patency (165), stimulation of the hypoglossal nerve to increase upper airway muscle tone has emerged as a potential therapy for OSA. HGNS involves surgical placement of a pacing electrode on the hypoglossal nerve along with a sensing electrode on an intercostal muscle to synchronize pacing to inspiration. Preliminary uncontrolled studies demonstrated that HGNS was feasible in improving OSA severity, sleepiness, and quality of life (166,167), but certain groups (those with concentric palatal collapse or morbid obesity) were less likely to respond. Several HGNS systems have been developed. One of these devices was found to be ineffective (168) but a second device produced significant improvements in AHI and sleepiness in a highly selected population (169).

Wake-promoting agents

Residual sleepiness is common even in CPAP compliant OSA patients. Among those using CPAP >6 hours/night, subjective sleepiness is noted in 22% and objective sleepiness in 52% (170). The wake-promoting agent, modafinil, when added to CPAP, improves both subjective and objective measures of sleepiness with a mean effect on ESS of 6-7 points. Although there is concern that modafinil use may lead to reductions in CPAP adherence, this effect has not been consistently observed in clinical trials (171-174). Modafinil is also effective as adjunctive therapy when CPAP is stopped for short periods of time (e.g., when traveling), preventing

re-emergence of sleepiness (175). In general, armodafinil produces similar results as modafinil (176,177).

Individualized treatment of disease

The traditional model of OSA pathophysiology is that of compromised upper airway anatomy leading to repetitive collapse during sleep (178,179). However, our understanding of the pathophysiology of OSA has broadened in recent years to include traits beyond compromised anatomy (180,181). At least three further physiological factors interact to play a role in OSA including (I) inadequate upper airway dilator muscle responsiveness (180,182); (II) an abnormal respiratory arousal threshold (183,184); and (III) a hypersensitive ventilatory control system (elevated loop gain) (185). The increasing recognition of the importance of these traits has led to the idea of an individualized approach to therapy, where the specific underlying mechanisms in each patient are addressed via novel focused therapies.

The upper airway dilator muscles play an important role in maintaining airway patency during both sleep and wakefulness (186). The largest of these muscles is the genioglossus, which is innervated by the hypoglossal nerve (187-190). Contraction of this muscle appears to be both necessary and sufficient to prevent pharyngeal collapse in patients with OSA (165). However, responsiveness of the genioglossus to respiratory stimuli such as negative pressure and hypercapnia during sleep is variable, and overall there is decreased descending neural stimulation to the dilator muscles at sleep onset (191). Ultimately some individuals demonstrate a robust response leading to maintenance of airway patency, and others an insufficient response (192-194).

Interestingly, awake OSA patients have higher electromyographic signals in the genioglossus compared to healthy controls, suggesting a compensatory response to airway vulnerability (195). The loss of this heightened neuromuscular stimulation at sleep onset is theorized to contribute to airway collapse in susceptible individuals (196). A particular subgroup of OSA patients has insufficient genioglossus responsiveness, perhaps suggesting an important role of this trait in apnea pathogenesis as well as providing a potential therapeutic target (197). Current understanding of the pathways leading to genioglossal activation is limited making targeted pharmacologic intervention difficult, though studies of tricyclic antidepressants (196,198), ampakines (199), and cholinesterase inhibitors (200) are ongoing. Other pharmacologic interventions are focused on increasing the arousal threshold (discussed below), which may allow for successive dilator muscle recruitment and subsequent airway opening prior to arousal. Finally, electrical stimulation of the hypoglossal nerve or the dilator muscles directly, as previously discussed, may be a viable intervention in some cases (201-204).

Another physiological trait of interest in OSA is the arousal threshold. When a stimulus increases to a level of intensity that it causes awakening from sleep, the arousal threshold has been reached. An individual with a low arousal threshold is awoken easily, and an individual with a high arousal threshold is relatively resistant to awakening. In regard to OSA, the major stimulus to arousal includes intrathoracic pressure which is a function of respiratory efforts during hypoxemia and hypercapnia (205). Arousal is believed to be an important defense mechanism to protect against severe hypoxemia, and an arousal threshold that is too high could contribute to tissue hypoxia during apnea (206). Conversely a low arousal threshold in the setting of OSA leads to repetitive awakenings prior to sufficient recruitment of airway dilator muscles (207,208). Each such awakening is associated with an acute ventilatory escalation due in part to hypercapnia and a concurrent drop in CO_2 setpoint (209,210). Recurrent surges in ventilation can in turn contribute to respiratory instability and further episodes of apnea and hypopnea, most dramatically in patients with elevated loop gain (described below) (208,211).

Although patients with OSA on average have a somewhat higher arousal threshold in comparison to controls, a low arousal threshold likely plays a role in disease pathophysiology in roughly one third of affected patients (183,197,207). For these patients an increase in arousal threshold, for example via pharmacologic intervention, may allow for recruitment of airway dilator muscles and avoidance of respiratory instability (208). Concerns related to such therapies include the possibility of blunting arousal in response to severe hypoxia as well as suppressing upper airway dilator muscle activity, thereby worsening apnea (212). The hypnotic trazodone, has been shown to increase arousal threshold to CO_2 in patients with OSA and a low arousal threshold, without impairing pharyngeal muscle activity or increasing airway collapsibility (206,212,213). Other non-myorelaxant sedatives such as triazolam and eszopiclone have demonstrated a similar effect on arousal threshold (214,215).

The respiratory control system is a loop made up of the lungs, circulating blood, chemoreceptors, and descending

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neurologic signals that manage ventilatory drive. The sensitivity of this system to changes in respiratory stimuli can be conceptualized using the engineering notion of loop gain, which represents the stability of a negative feedback loop (216). In relation to respiratory control, loop gain is defined as the ratio of a ventilatory response over the perturbation in ventilation. In a system with high loop gain, a small change in respiratory stimuli (e.g., hypercapnia) leads to an exaggerated ventilatory response. This overcorrection can lead to respiratory instability (self-sustaining oscillations). In contrast, a system with low loop gain is likely to maintain respiratory stability (185,217). There are three major components that affect loop gain. The first is 'plant gain', or changes in the respiratory apparatus including the lungs and circulating blood in response to neuronal stimulation. The second is 'controller gain', or the sensitivity of chemoreceptors to oxygen and CO_2 levels and the subsequent output from respiratory centers eliciting a response from the plant. The third is the circulation delay between the plant (lungs) and controller (chemoreceptors), which leads to a lag in response to changes in blood gases.

Not all OSA patients have an abnormal loop gain. However, in a subgroup of individuals with OSA and only mild airway collapsibility, loop gain was nearly 50% greater in comparison to controls (197). This finding suggests that loop gain may play a role in OSA pathophysiology in a subset of patients, and offers a possible therapeutic target. Of the three components, only controller gain has been shown to be elevated in OSA patients (185,217,218). Several therapies impacting loop gain are being evaluated as potential treatments for OSA. Supplemental oxygen decreases loop gain in patients with high baseline loop gain via reduction in controller gain, without significantly affecting pharyngeal collapsibility, upper airway responsiveness, or arousal threshold (219,220). In contrast, the carbonic anhydrase inhibitor acetazolamide lowers loop gain (regardless of baseline levels) primarily via reduction in plant gain, likely due to a lowering of $PaCO_2$ (221).

Conclusions

The most effective and reliable treatment for OSA today remains CPAP, whose main limitation is tolerability resulting in suboptimal patient adherence. The coming decades will demand new interventions to augment CPAP adherence and alternative therapies tailored to the individual patient. Even without newer options, further

research is required to individualize treatment options optimally.

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Footnote

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Obstructive sleep apnea is a common disorder in the population—a review on the epidemiology of sleep apnea

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Abstract: The prevalence of obstructive sleep apnea (OSA) defined at an apnea-hypopnea index (AHI) ≥5 was a mean of 22% (range, 9-37%) in men and 17% (range, 4-50%) in women in eleven published epidemiological studies published between 1993 and 2013. OSA with excessive daytime sleepiness occurred in 6% (range, 3-18%) of men and in 4% (range, 1-17%) of women. The prevalence increased with time and OSA was reported in 37% of men and in 50% of women in studies from 2008 and 2013 respectively. OSA is more prevalent in men than in women and increases with age and obesity. Smoking and alcohol consumption are also suggested as risk factors, but the results are conflicting. Excessive daytime sleepiness is suggested as the most important symptom of OSA, but only a fraction of subjects with AHI >5 report daytime sleepiness and one study did not find any relationship between daytime sleepiness and sleep apnea in women. Stroke and hypertension and coronary artery disease are associated with sleep apnea. Cross-sectional studies indicate an association between OSA and diabetes mellitus. Patients younger than 70 years run an increased risk of early death if they suffer from OSA. It is concluded that OSA is highly prevalent in the population. It is related to age and obesity. Only a part of subjects with OSA in the population have symptoms of daytime sleepiness. The prevalence of OSA has increased in epidemiological studies over time. Differences and the increase in prevalence of sleep apnea are probably due to different diagnostic equipment, definitions, study design and characteristics of included subjects including effects of the obesity epidemic. Cardiovascular disease, especially stroke is related to OSA, and subjects under the age of 70 run an increased risk of early death if they suffer from OSA.

Keywords: Epidemiology; population-based; sleep apnea; prevalence

Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of partial and complete airway obstructions during sleep with repetitive apneas and hypopneas as a result (1). The disease severity is measured using the apnea-hypopnea index (AHI), i.e., the mean number of apneas and hypopneas per hour of sleep. OSA is defined when the AHI is ≥5 and OSA syndrome when AHI ≥5 is accompanied with daytime sleepiness (1).

The American Association of Sleep Medicine defined daytime sleepiness as mild, moderate and severe in relation to impact on social life during the daytime (1). The Epworth Sleepiness Scale (ESS) is, however, the most often used measure to define daytime sleepiness (2,3). Diagnostic equipment and definitions of oxygen desaturations, apnea, hypopnea, OSA and daytime sleepiness has, changed over time, which in turn affects estimates of the prevalence of sleep apnea.

In the first prevalence surveys when sleep apnea was considered a rare disorder, sleep recordings were only performed in sub-samples with a high risk of OSA in a first-stage screening procedure, and the estimated prevalence in the whole population was based on the assumption that there was no OSA at all among the remaining participants. The estimated prevalence of OSA syndrome in these studies ranged from 0.7% to 3.3% (4-8).

Patients in sleep clinic cohorts have all been referred for the diagnostic sleep test because of symptoms suggestive of the diagnosis and they are most frequently heavy snorers suffering from daytime sleepiness. Epidemiological studies on sleep apnea will identify all subjects with OSA defined as AHI ≥5. However, only part of them will have symptoms such as snoring and daytime sleepiness reflecting subjects eligible for sleep apnea investigation on clinical grounds. This article reviews the epidemiology of OSA on prevalence and associated factors including possible risk factors and consequences.

Prevalence of OSA

We identified eleven population-based epidemiological studies from US, Chine, Spain, India, Korea, Japan and Sweden published between 1993 and 2013. All eleven studies were done in two stages. In stage one, they send postal questionnaires to a random sample of the population. In stage two, they investigated a random sample of responders from stage one, with oversampling of subjects who reported snoring and daytime sleepiness and then weighted their results to the population. The prevalence of OSA defined at an AHI ≥5 were a mean of 22% (range, 9-37%) in men and 17% (range, 4-50%) in women (*Table 1*, *Figure 1*) (3,8-17). OSA syndrome defined as apnea-hypopnea index ≥5 and excessive daytime sleepiness occurred in 6% (range, 3-18%) of men and in 4% (range, 1-17%) of women (*Figure 1*). The prevalence in different studies has increased with time and OSA in the last studies was reported in 37% of men and in 50% of women (3,16). The differences over time could be due to different equipment and definitions for the apnea-hypopnea scoring. There are also differences in study design and populations. The results may also be affected by an increased amount of obese subject due to the obesity epidemic.

Associated factors with OSA

Gender

OSA is more common in men than women. The male-

to-female ratio is estimated to about 2:1 in the general population (*Table 1*, *Figure 1*) and the prevalence of snoring shows similar gender differences (18-20). The male predominance is higher in clinical populations (21,22). Possible explanations for the male predominance include hormonal effects on upper airway muscles and collapsibility, gender differences in body fat distribution and differences in pharyngeal anatomy and function. Hormonal influences could play an important role in the pathogenesis of OSA, as the prevalence seems to be higher in post- versus pre-menopausal women (17,23). The pathophysiological roles of hormones are, however, unclear and the gender differences in prevalence remained also in the elderly (24).

A recent study, by Franklin *et al.*, reported that sleep apnea occurs in as much as 50% of females aged 20-70 years old in the population (3). There was no relationship between OSA and daytime sleepiness in this study. Instead hypertension, obesity and age were associated with sleep apnea in females. It is, thus, possible that sleep apnea has not been observed as a public heath problem in females, as they have other signs of sleep apnea than males.

Age

Snoring frequency increases with age up to 50 to 60 years old and then decrease in both men and women (6,18,25,26). The prevalence of OSA also increases with age independent of other risk factors including obesity (3,8,27,28). On the contrary to snoring, the prevalence of OSA still increases also after the age of 60 years (3,8,10). Bixler *et al.* reported an increase in OSA after 65 years but the frequency of OSA syndrome declined (8). The above findings indicate that self-reported snoring and doctor-diagnosed OSA syndrome display similar age distributions with a decline at older ages in contrast to the age distribution of OSA with an AHI over five that increase with age also in the elderly.

Several studies have reported little or no association between sleep-disordered breathing and morbidity and mortality at older ages, and it has been suggested that sleep apnea in seniors represents a specific entity compared with middle-aged adults (29).

Obesity

Obesity is a major risk factor for snoring and sleep apnea and a majority of patients with OSA are overweight (3,30-32). Caloric restriction or bariatric surgery reduces the severity of sleep apnea (28,33-36). One randomized controlled study

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Table 1 Population-based studies on the prevalence of obstructive sleep apnea (OSA) and OSA syndrome

Ref./study population	Sample size gender and criteria	Estimated prevalence of AHI ≥5 (%)	Estimated prevalence of AHI ≥15 (%)	Estimated prevalence of OSA syndrome (%)	Methodology	Hypopnea definition
Young *et al.*, 1993 (9)						
Random sample of 3,513 state employees in Wisconsin, USA; age: 30-60 years	M: 352 W: 250 Habitual snorers (n=355) and a random sample of non-habitual snorers	M: 24 W: 9	M: 9 W: 4	M: 4.0 W: 2.0	Attended PSG	Discernible reduction in airflow and ≥4% oxygen desaturation
Bixler *et al.*, 1998 (8)						
Random sample of 4,364 men in Pennsylvania, USA; age: 20-100 years	M: 741 Age-stratified cohorts. Oversampling of high-risk individuals	M: 17	M: 7	M: 3.3	Attended PSG	Discernible reduction in airflow and ≥4% oxygen desaturation
Durán *et al.*, 2001 (10)						
2,148 subjects from the general population in Vitoria-Gasteiz, Spain; age: 30-70 years	M: 325 W: 235 Tentative diagnosis of OSAH (n=390) and a random sample	M: 26 W: 28	M: 14 W: 7	M: 3.4 W: 3.0	Attended PSG	50% airflow reduction and either ≥4% oxygen desaturation or an EEG arousal
Ip *et al.*, 2004 (11,12)						
Male (n=1,542) and female (n=1,532) office workers in Hong Kong, China; age: 30-60 years	M: 153 W: 106	M: 8.8 F: 3.7	M: 5.3 W: 1.2	M: 4.1 W: 2.1	Attended PSG	Discernible reduction in airflow and ≥4% oxygen desaturation
Udwadia *et al.*, 2004 (13)						
658 healthy men coming to hospital for routine health check in Bombay, India; age: 35-65 years	M: 250 All snorers (n=171) and 25% of non-snorers	M: 19.5	M: 8.4	M: 7.5	Home PSG	Discernible 50% reduction in airflow and ≥4% oxygen desaturation
Kim *et al.*, 2004 (14)						
Population-based sample of 5,020 residents in Seoul, Korea; age: 40-69 years	M: 309 W: 148 Oversampling of habitual snorers	M: 27 W: 16	M: 10.1 W: 4.7	M: 4.5 W: 3.2	Home or in laboratory PSG	Discernible reduction in airflow and ≥4% oxygen desaturation

Table 1 (*continued*)

Table 1 (*continued*)

Ref./study population	Sample size gender and criteria	Estimated prevalence of AHI ≥5 (%)	Estimated prevalence of AHI ≥15 (%)	Estimated prevalence of OSA syndrome (%)	Methodology	Hypopnea definition
Sharma *et al.*, 2006 (15)						
2,400 citizens in Delhi, India; exclusion criterion: several diseases; age: 30-60 years	M: 88 W: 63 Habitual snorers (n=77) and non-snorers	M: 19.7 W: 7.4	n/a	M: 4.9 W: 2.1	Attended in laboratory PSG	Discernible 50% reduction in airflow and ≥4% oxygen desaturation
Nakayama-Ashida *et al.*, 2008 (16)						
466 male employees of a wholesale company in Osaka, Japan; age: 23-59 years	M: 322	M: 37.4	M: 15.7	M: 17.6	Home type 3 portable monitors and actigraphy	≥50% reduction in nasal pressure or respiratory effort and ≥3% oxygen desaturation
Franklin *et al.*, 2013 (3)						
Population-based sample of 10,000 women in Uppsala, Sweden; age: 20-70 years	W: 400 Oversampling of habitual snorers	W: 50	W: 20	W:17	Home PSG	≥50% reduction in oro-nasal thermistor and nasal pressure, and ≥3% oxygen desaturation

OSA, obstructive sleep apnea; OSAH, obstructive sleep apnea hypopnea; PSG, polysomnography; M, men; W, women.
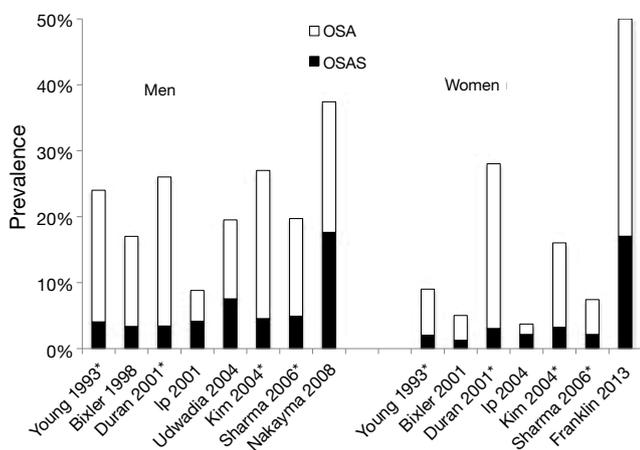
Figure 1 Prevelence of OSA = AHI >5 and OSAS AHI >5+ daytime sleepiness men and women in different populations. OSA, obstructive sleep apnea; *, men and women in the same publication.

reported a decrease in AHI using very low calorie diet (37). Another recent study reported that despite an effect of diet on AHI compared with continuous positive airway pressure (CPAP), patients were still better off with the combination of diet and CPAP than with CPAP alone (38). Men are more likely than women to increase their AHI at a given weight gain regardless of starting weight, waist circumference, age, or ethnicity (39).

Obesity is believed to predispose to OSA because of mass loading in the upper airway (40). Controversy remains whether specific measures of body habitus, such as neck or waist circumference, are better predictors of sleep-disordered breathing as compared with body mass index (BMI) alone. Neck circumference was in a population-based sample more important as a risk factor for snoring with increasing BMI in obese than in lean women (26).

Young *et al.* estimated that 58% of moderate to severe cases of OSA is due to a BMI of ≥25 kg/m^2 (34). This

highlights the need for effective strategies to implement long-term weight-loss programs to prevent OSA and the ongoing epidemics of obesity. Not only subjects with obesity and fat necks suffer from sleep apnea, but also lean subject and about one-third of OSA syndrome patients are non-obese (41). Franklin *et al.* reported that 39% of normal weighted women had OSA but only 0.1% of them had severe sleep apnea (3).

Smoking

Several cross-sectional epidemiological surveys observed significant associations between cigarette smoking and snoring or sleep apnea (18,19,42-46). Possible underlying mechanisms include airway inflammation and sleep instability from overnight nicotine withdrawal (47). Never-smokers who have been exposed to passive smoking on a daily basis display an increase in the odds of being a habitual snorer of 1.6 (95% CI, 1.2-2.1) after adjusting for age and BMI according to the Respiratory Health in Northern Europe Study (44). In a Swedish longitudinal study, smoking predicted the development of snoring in men younger than 60 years old but not in older ones (25).

Wetter *et al.* found a dose-response relationship between smoking and the severity of sleep apnea. Heavy smokers ran the greatest risk, while former smoking was unrelated to snoring and sleep-disordered breathing after adjustment for confounders (46). Smoking is, however, not an established risk factor for OSA. In the analysis from the Sleep Heart Health Study, smokers actually displayed less sleep apnea than non-smokers and there are still no available data on the impact of smoking on the incidence and remission of sleep apnea (48).

Alcohol

Alcohol intake reduces motor output to the upper airways with hypotonia of the oropharyngeal muscles as a result (49). In studies performed in the laboratory, alcohol increases both the number of apneas and the duration of apnea (50,51). The results did, however, diverge, when the relationship between chronic alcohol use and snoring or sleep apnea was analyzed in epidemiological studies and an association was found by some but not by others (13,25,30,52-54). Svensson *et al.* reported that alcohol dependence was only related to snoring in lean women with a BMI of <20 kg/m^2 (26). It is thus possible that the alcohol-induced reduction in motor output to the upper airways is more important in lean women without compromised upper airways from fat deposits and overweight.

Excessive daytime sleepiness

Excessive daytime sleepiness is regarded as the most common and most important symptom of OSA. Numerous randomized controlled trials have demonstrated a significant improvement in daytime sleepiness when such patients are effectively treated with CPAP as compared to sham CPAP or oral placebo (55-60).

Daytime sleepiness is related to OSA and snoring in the general population studies (9,61-63). In the Wisconsin Sleep Cohort Study, about 23% of the women with an AHI of ≥5 reported excessive daytime sleepiness compared with only 10% of non-snoring women (9). The corresponding prevalence in men was 16% and 3% respectively. Similar findings were reported from the Sleep Heart Health Study using the ESS with a significant, progressive increase in sleepiness with increasing AHI in both older and younger subjects and independent of gender, age and BMI (61).

The evidence of apnea induced daytime sleepiness is, however, weak as only a fraction of patients with OSA in the population report daytime sleepiness. Attempts to find the suggested association between the arousals and sleepiness have also failed (64,65). Daytime sleepiness can be due to a number of factors and OSA patients may have suffered from other disorders of sleepiness than sleep apneas. Svensson *et al.* reported that snoring, but not sleep apnea (AHI >15) was related to excessive daytime sleepiness (63). The association between OSA and sleepiness is also less evident in patients with chronic disease such as in patients with congestive heart failure who report less daytime hypersomnolence regardless of whether they have OSA or not (66). Sleepiness is also frequently reported in the absence of OSA in elderly people and in patients with end-stage renal disease (67,68).

Hypertension

Sleep apnea and hypertension are both prevalent in the community and many individuals suffer from both. Several large population-based, cross-sectional studies reported an independent association between the two conditions (3,10,69-72). Self-reported snoring is also a predictor of developing hypertension in both males and females (43,73,74). Peppard *et al.* analyzed the odds ratios for the presence of hypertension at a 4-year follow-up among 709 middle-aged participants in the Wisconsin cohort, all of

who had been investigated with polysomnography at baseline. Compared with subjects with no OSA, the adjusted odds ratio for prevalent hypertension at follow-up was 2.03 (95% CI, 1.29-3.17) for mild OSA (AHI, 5-14.9) and 2.89 (95% CI, 1.46-5.64) for moderate to severe OSA (AHI ≥15) (75). The same group also provided data from a sub-group who were followed-up after a mean of 7 years using 24-hour blood pressure studies. Regardless of confounders including baseline blood pressure and progress of sleep apnea, there was a significant dose-response relationship between the severity of sleep apnea at baseline and the risk of developing systolic non-dipping blood pressure during sleep (76).

The impact of snoring and OSA on hypertension is less pronounced in overweight and obese subjects when compared with normal-weights in population-based samples (43,69,71). Analyzed by age group, there is an independent relationship of snoring or OSA on hypertension among young and middle-aged participants, but not in the elderly (71-73,77,78). An AHI of ≥15 was independently associated with hypertension in subjects aged <60 years, with an adjusted odds ratio of 2.38 (95% CI, 1.30-4.38), among 6,120 participants in the Sleep Heart Health Study, while no such relationship was found between sleep apnea and hypertension among subjects above that age (72).

Although observational studies indicate a causal relationship between OSA and hypertension the effectiveness of reducing blood pressure by treating OSA is less clear and intervention studies using CPAP have produced mixed results (79).

Coronary artery disease

OSA frequently coexists, but is usually being undiagnosed in patients with cardiovascular disease and several cross-sectional studies support a strong association between OSA and prevalent coronary artery disease, defined as myocardial infarction and/or angina pectoris (80-82). However, sleep apnea was assessed after coronary artery disease was established in the cited studies and thereby limits the conclusion on an etiologic relationship. Cross-sectional epidemiologic studies on self-reported coronary artery disease and snoring or objectively measured OSA have reported a positive association, although of considerably smaller magnitude than that observed in case-control studies (18,83). Among 6,424 participants who underwent in-home polysomnography in the Sleep Heart Health Study, Shahar *et al.* reported that subjects with the highest quartile of AHI >11 had an adjusted odds ratio of 1.27 of self-reported coronary artery disease after adjusting for confounders including hypertension (84). The relative high age with a mean of 64 years old of participants at study start could be an explanation to the rather modest association.

Patients with OSA had a higher incidence of coronary artery disease (16.2%) compared with snorers without OSA (5.4%) in a prospective study over 7 years (85). Efficient treatment with CPAP significantly reduced the risk of adverse cardiovascular outcomes both when it comes to primary and secondary prevention (85-87).

Population-based prospective studies on sleep apnea and incidence of coronary artery disease are still lacking.

Stroke

Clinical cohorts suggest an important link between sleep apnea and stroke. Spriggs *et al.* followed patients with recent stroke until death or 6 months and found that previous stroke and regular snoring were the only two risk factors that adversely affected mortality (88). Yaggi *et al.* followed 1,022 patients being investigated on clinical grounds an concluded that OSA syndrome significantly increases the risk of stroke or death from any cause, and the increase is independent of other risk factors, including hypertension (89). Valham *et al.* found a dose-response relationship between AHI at baseline among patients with coronary artery disease and the incidence of stroke during a 10-year follow-up after adjusting for potential confounders (90). Moreover, in stroke survivors, the occurrence of OSA, but not central sleep apnea, was a significant predictor of early death (91).

Population based studies also support the evidence of stroke due to OSA. Munoz *et al.* reported that severe sleep apnea (AHI ≥30) at baseline was associated with a significantly increased risk of developing an ischemic stroke (adjusted hazard ratio 2.52, 95% CI, 1.04-6.01) from a 6-year longitudinal study of a population-based cohort, initially event-free subjects aged 70-100 years (92). Arzt *et al.* investigated a younger population-based cohort of 1,189 subjects, mean age 47 years with polysomnography. During the following 4 years, 14 subjects suffered a first-ever stroke and this was related to sleep apnea defined as an AHI of ≥20 at baseline, although the association did not reach statistical significance after adjusting for age, gender and BMI (adjusted OR 3.08, 95% CI, 0.74-12.81) (93).

Diabetes mellitus

Sleep-disordered breathing and diabetes mellitus share several

Table 2 Population-based studies designed to investigate the relationship between sleep-disordered breathing and mortality

Population/ref.	n	Adjusted confounders	Marker of SDB	Adj hazard ratio; all-cause mortality	Comments
Sample of men in Uppsala, Sweden; follow-up period: 10 yrs (114)	3,100 30-69 yrs	Age, BMI, hypertension heart disease, diabetes	No snoring or EDS Snoring, no EDS EDS, no snoring Snoring and EDS	1 1.1 (0.8-1.5) 1.1 (0.6-1.9) 1.8 (1.2-2.5)	In stratified analyses: significant only in men <60 yrs [adj HR 2.7, (range, 1.6-4.5)]
Wisconsin sleep cohort; follow-up period: 18 yrs (23)	1,522 30-60 yrs	Age, gender, BMI	AHI 0-<5 AHI 5-<15 AHI 15-<30 AHI ≥30	1 1.6 (0.9-2.8) 1.4 (0.6-3.3) 3.0 (1.4-6.3)	No sign interaction with age, gender or EDS
Sleep Heart Health Study Average; follow-up 8.2 yrs (115)	6,441 Mean age 62.9±11.0	Age, race, BMI, gender, smoking, diabetes, blood pressure, cardiovascular disease	AHI 0-<5 AHI 5-<15 AHI 15-<30 AHI ≥30	1 0.93 (0.80-1.08) 1.17 (0.97-1.42) 1.46 (1.14-1.86)	In stratified analyses the adj hazard ratio was only significant in men <70 [adj HR 2.09 (range, 1.31-3.33)]

Adj, adjusted; EDS, excessive daytime sleepiness.

risk factors. Insulin resistance and/or type 2 diabetes mellitus coexist with snoring or sleep apnea in general population cross-sectional studies independent of obesity and other confounders (94-101). Furthermore, an independent association between self-reported snoring and incident diabetes is reported in both males and females (102,103).

Longitudinal studies on OSA as a risk factor for future diabetes mellitus have not been conclusive. Among 1,387 participants in the Wisconsin Sleep Cohort, subjects with an AHI ≥15 did not differ significantly from those with an AHI of <5 when it came to the risk of developing diabetes mellitus over a 4-year period (OR 1.62; 95% CI, 0.7-3.6) when adjusting for age, gender, and body habitus (99). Similar findings were reported from the Busselton health study (104). On the contrary, Botros *et al.* found an independent association between sleep apnea at baseline and incident diabetes in an observational cohort study including 1,233 consecutive patients without diabetes (105). Also in a long-term follow-up of a community-based sample of men there was an independent association between oxygen desaturation index >5 at baseline and incident diabetes mellitus at follow-up after 11 years (OR 4.4; 95% CI, 1.1-18.1), after adjusting for age, BMI, and hypertension at baseline and delta BMI and years with CPAP during follow-up (106).

Mortality

Clinic-based studies suggest that patients with OSA syndrome have a higher mortality risk (107) and that

treatment with tracheostomy or CPAP attenuates this risk (108-110). The lack of randomized, controlled interventional trials clearly limits the evidence, as non-treated patients have either been non-compliant with prescribed therapy or have for some reason not been selected for effective treatment. Clinical mortality studies might also be biased, as patients under treatment for some other serious morbidity might also be more likely to be referred for an evaluation of sleep apnea, leading to an overestimation of mortality.

The results diverge in studies investigating whether patients with OSA syndrome have a shorter survival or not. No increased mortality rate was found between apnea-hypopnea scores in two prospective studies investigating elderly populations (111,112), while a significant association was seen in another study, but in women only (113). Lavie *et al.* in a prospective study found that the apnea index was a predictor of excess mortality in the fourth and fifth decade but not in elderly men (107). This is in accordance with the results from of a population-based study from Uppsala in Sweden where men aged 30-69 years were investigated by postal questionnaire and followed over 10 years (114). Snoring men reporting excess daytime sleepiness had a significant increase in mortality, but the age-adjusted relative risk decreased with increasing age and was no longer significant after age 50 years. Snoring alone had no impact on mortality in any of the age groups (*Table 2*).

The impact of OSA on mortality in population-based cohorts has recently been analyzed in the Wisconsin Study (23), and in the Sleep Heart Health Study (115), and both

reported an increased mortality rate with increasing severity of sleep apnea (*Table 2*). Subjects with an AHI ≥30 had an adjusted hazard ratio for all-cause mortality of 3.0 (95% CI, 1.4-6.3) and 1.46 (95% CI, 1.14-1.86) respectively, compared with those with an AHI of <5. Similar results were obtained for cardiovascular mortality in both studies and the exclusion of subjects treated for sleep apnea did not change the results. However, the adjusted hazard ratios for severe sleep apnea only remained significant in younger men <70 years in the Sleep Heart Health Study.

Conclusions

OSA is highly prevalent in the population. It is related to age and obesity. Only a part of subjects with OSA in the population have symptoms in the form of daytime sleepiness. The prevalence of OSA and OSA syndrome has increased in epidemiological studies over time. Differences and the increase in prevalence of sleep apnea are probably due to different diagnostic equipment, definitions, study design and characteristics of included subjects. Cardiovascular disease, especially stroke is related to OSA and subjects under the age of 70 run an increased risk of early death if they suffer from OSA.

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Footnote

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Patients' preference of established and emerging treatment options for obstructive sleep apnoea

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Background: Obstructive sleep apnoea (OSA) is the most common form of sleep-disordered breathing. The standard treatment, continuous positive airway pressure (CPAP), has limited long-term compliance. Alternative treatment options are required and new methods, including hypoglossal nerve stimulation (HNS) and continuous transcutaneous electrical stimulation (CTES), are currently emerging. We report on patients' preference for different treatments of OSA.

Methods: We recorded patients' age, gender, body mass index (BMI), Epworth Sleepiness Scale (ESS), Functional Outcomes of Sleep Questionnaire with 10 questions (FOSQ10), severity of OSA, and current treatment. We showed pictures of existing [CPAP, mandibular advancement device (MAD)] and emerging treatments (HNS and CTES). We then asked (I) whether participants were interested in further information about HNS/CTES; (II) if they would be willing to try HNS/CTES; and (III) if they were to choose only one of the four listed treatments, which one would they prefer to use every night.

Results: One hundred sixty-two patients completed the survey {81 males, mean age 52 [12] years, BMI 34 [7] kg/m^2, ESS 10.2 (6.0) points, FOSQ10 28.5 (8.1) points}. The majority of the respondents (89.5%) had been diagnosed with OSA. A total of 91.3% of the respondents were interested in more information and were willing to try HNS/CTES. Most respondents preferred the potential use of CTES (56.7%), while 21.7% chose HNS, 17.8% CPAP, and 3.8% the MAD. There were no differences in the characteristics of the patients who preferred CTES compared to those who preferred other treatments, but a regression analysis revealed that a low ESS score was an independent predictor of patients choosing CTES (P<0.05).

Conclusions: More than 9 out of 10 of the respondents were interested in trying emerging technologies to treat OSA, most preferring CTES. Less sleepy patients were more likely to choose less invasive treatments. These findings will likely impact on future research and development of therapies for sleep-disordered breathing.

Keywords: Sleep apnoea; continuous positive airway pressure (CPAP); sleepiness; electrical stimulation

Introduction

Obstructive sleep apnoea (OSA) is the most common form of sleep-disordered breathing, affecting up to 10% of middle-aged men and 3% of middle-aged women (1).

It is characterised by intermittent and repeated episodes of upper airway obstruction during sleep and can result in irregular breathing at night and excessive sleepiness during the day (2). OSA is also associated with several co-

morbidities, including hypertension (3), ischaemic heart disease (4), stroke (5), congestive heart failure (6), obesity (7), and diabetes (8).

Permanent tracheostomy was the first effective therapy for OSA, and it was the most common treatment used for OSA in the 1970's and early 1980's (9). The management of OSA was revolutionized in 1981 by the introduction of continuous positive airway pressure (CPAP), which can completely prevent upper airway occlusion during sleep in patients with OSA (10). In a subsequent study done 2 years later, Sullivan *et al.* also showed that CPAP is an effective treatment for patients with severe obesity-hypoventilation syndrome and can lead to remission of the underlying disordered breathing during sleep (11). Since its development in the 1980's, CPAP has been shown to reduce the symptoms of OSA, improve the risk for associated co-morbidities, especially arterial hypertension, and potentially impact on mortality in patients with severe OSA (12,13).

According to current guidelines, CPAP therapy is the standard treatment in patients with moderate to severe OSA (14). The effectiveness of CPAP therapy, however, is dependent on patients' adherence and symptomatic response to the treatment (15). At the least, one third of all patients initiated on CPAP therapy do not use the treatment at 5 years. Long-term CPAP use can be predicted by the uptake within the first 3 months and is dependent on disease severity and daytime sleepiness (16).

Because of the limited long-term compliance to CPAP, alternative treatments for OSA are required for those patients that fail to comply or respond to CPAP therapy. Mandibular advancement devices (MADs) have been developed to reposition the jaw and keep the upper airway patent during sleep. These devices have been shown to reduce the severity of OSA and improve daytime sleepiness. MADs are currently recommended for the treatment of mild OSA (17).

Since 2011, electrical stimulation of the upper airway dilator muscles has emerged as a potential alternative to CPAP, as upper airway patency is correlated with the electromyographic activity of the genioglossus muscle. Strollo *et al.* have shown that unilateral stimulation of the hypoglossal nerve by a surgically implanted neuro-stimulator device significantly reduces the severity of OSA, daytime sleepiness, and improves quality of life (18). Consequently, hypoglossal nerve stimulation (HNS) has recently been approved by the US Food and Drug Administration (FDA) to treat moderate to severe OSA (19).

HNS, however, involves the surgical implantation of a neuro-stimulator and is highly expensive. A non-invasive form of electrical stimulation could be advantageous in that it offers to assess patients with limited risk and at low costs. Continuous transcutaneous electrical stimulation (CTES) involves low-current stimulation of the genioglossus muscle. CTES has been shown to reduce ventilatory load and neural drive in patients with OSA to a similar degree as HNS (20). The efficacy of CTES as a treatment option for OSA, however, is still being evaluated. The current published studies on the non-invasive use of electrical stimulation have revealed varying results, which is likely to reflect the lack of standardisation of the approach (21).

The low compliance remains the significant limitation of CPAP as a long-term treatment for OSA. It is crucial that patients can tolerate any long-term treatment for OSA, and in this overview we present data on treatment preferences of patients with OSA and discuss the implications.

Methods

We performed face-to-face interviews in outpatient and inpatient clinics in the Sleep Disorders Center and Lane Fox Respiratory Unit at Guy's and St. Thomas' NHS Foundation, London, UK in addition to using a web platform supported by a patients' group to distribute a survey (local registration number 2014-4621).

In the survey, we recorded patients' age, gender, body mass index (BMI), Epworth Sleepiness Scale (ESS), Functional Outcomes of Sleep Questionnaire with 10 questions (FOSQ10), diagnosis of OSA, and the current treatment.

We showed pictures of existing treatments (CPAP, MAD) and emerging treatments for OSA (HNS and CTES). The survey concluded by asking:

(I) Whether patients would like to receive more information about HNS and CTES;

(II) If the participants would be willing to try out HNS or CTES;

(III) If they had to choose one of the four listed treatments (CPAP, MAD, HNS, CTES) to use every night to treat their OSA, which would they prefer to use.

Participants were given the one page survey along with the pictures of the CPAP, MAD, HNS, and CTES. The online survey contained the same questions and pictures as the survey that was distributed in person. We did not answer questions about the survey or treatments in order to standardise the approach between face-to-face interviews and online participants. A link to the online survey was

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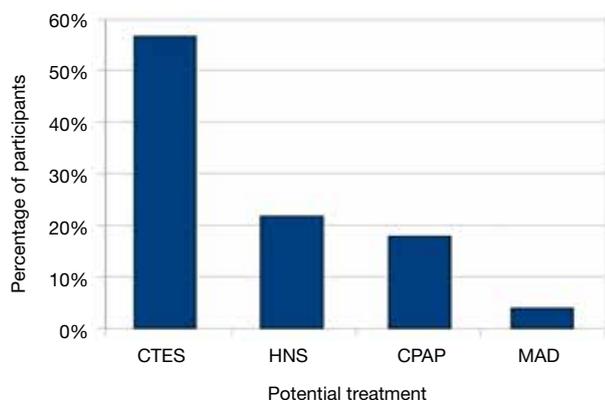
Figure 1 Patients' preferences for potentially available treatments for OSA. CTES, continuous transcutaneous electrical stimulation; HNS, hypoglossal nerve stimulation; CPAP, continuous positive airway pressure; MAD, mandibular advancement device; OSA, obstructive sleep apnoea.

Table 1 Main characteristics of the subgroups of interviewed patients

Items	All patients (n=162)	Online survey (n=136)	Face-to-face interview (n=26)	P value
Gender (male/female)	81/81	64/72	17/9	0.133
Age (years)	52 [12]	51 [11]	58 [14]	0.011
Height (m)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	0.100
Weight (kg)	98.8 (23.2)	99.8 (22.7)	92.8 (26.2)	0.135
BMI (kg/m^2)	33.9 (7.3)	34.4 (7.3)	30.7 (7.1)	0.056
ESS (points)	10.1 (5.9)	10.1 (6.0)	10.6 (5.6)	0.830
FOSQ10 (points)	28.5 (8.1)	27.9 (8.3)	31.5 (5.8)	0.097

BMI, body mass index; ESS, Epworth Sleepiness Scale; FOSQ10, Functional Outcomes of Sleep Questionnaire with 10 questions.

distributed by one of our expert patients at Guy's and St. Thomas' NHS Foundation Trust.

Statistical analysis

Data were analysed using GraphPad Prism (Version 5.02, GraphPad Software Inc., San Diego, California, USA) and SPSS statistics 20 (IBM, New York, USA). Data are presented as mean (standard deviation, SD) unless otherwise indicated. When we compared the data of the two subgroups of patients who underwent a face-to-face or an online interview, we used *t*-tests for continuous

variables and Fisher's exact test for categorical variables. We divided our sample of patients according to treatment preferences, and data were further analysed using a one-way ANOVA with a Kruskal-Wallis test and Dunn's correction for multiple test comparisons. Lastly, logistic regression was employed to identify correlations between independent variables (gender, age, BMI, FOSQ10, ESS, OSA severity) and treatment preference for CTES as the dependent variable. A level of significance was defined as P<0.05.

Results

A total of 136 patients filled out the online survey {64 males, mean age 51 [11] years, BMI 34.4 (7.3) kg/m^2, ESS 10.1 (6.0) points, FOSQ10 27.9 (8.3) points} and 26 patients completed the survey in a face-to-face interview {17 males, mean age 58 [14] years, BMI 30.7 (7.1) kg/m^2, ESS 10.6 (5.6) points, FOSQ10 31.5 (5.8) points}. The online group and face-to-face interview group were similar in terms of gender, BMI, daytime sleepiness, and quality of life related to OSA; however, patients in the face-to-face interview group were older than the patients in the online group (*Table 1*).

We combined the online and face-to-face interview groups to analyse the remaining data. The majority of the respondents (89.5%) had been diagnosed with OSA, and 46.9% of the patients had severe OSA. A total of 91.7% of the patients diagnosed with OSA were on treatment, while 8.3% were not receiving any treatment. A total of 95.4% of the respondents who received treatment were using CPAP, while 1.5% used MAD, and 3.1% used an alternative form of treatment. A total of 91.3% of all respondents were interested in more information and were willing to try emerging treatments, HNS or CTES. Most respondents (56.7%) preferred the potential use of CTES as a treatment for OSA, while 21.7% chose HNS, 17.8% preferred CPAP, and 3.8% the MAD (*Figure 1*). There were no differences between the patients who preferred different treatments in terms of age, BMI, gender, quality of life, and OSA severity (*Table 2*). A regression analysis included age, gender, OSA severity, quality of life, and daytime sleepiness as possible predictors of CTES preference. The analysis showed that a low ESS score was an independent predictor of patients preferring CTES (P<0.05, *Table 3*).

Discussion

Over 90% of patients, most of them treated with CPAP, were interested in more information and were willing to

Table 2 Characteristics according to preferred treatments

Items	CTES (n=75)	HNS (n=32)	CPAP (n=20)	P value
Gender (M/F)	37/38	15/17	9/11	0.932
Age (years)	53 [12]	51 [9]	48 [11]	0.225
BMI (kg/m^2)	34.3 (7.7)	34.5 (6.5)	35.3 (7.8)	0.896
ESS (points)	9.1 (6.0)	11.6 (5.4)	11.2 (6.4)	0.056
FOSQ10 (points)	28.5 (8.7)	26.9 (7.8)	27.9 (7.8)	0.576
OSA severity				
Mild OSA (%)	12.6	13.3	11.1	0.975
Moderate OSA (%)	28.5	30.0	38.8	0.702
Severe OSA (%)	58.7	56.6	50.0	0.805

CTES, continuous transcutaneous electrical stimulation; HNS, hypoglossal nerve stimulation; CPAP, continuous positive airway pressure; M, male; F, female; BMI, body mass index; ESS, Epworth Sleepiness Scale; FOSQ10, Functional Outcomes of Sleep Questionnaire with 10 questions; OSA, obstructive sleep apnoea.

Table 3 Multiple regression analysis to determine patient's treatment preferences

Items	Coefficients[a]			t	Sig.
	Unstandardized coefficients		Standardized coefficients		
	B	Std. error	Beta		
Constant	0.663	0.371		1.787	0.077
Age	0.008	0.004	0.173	1.805	0.074
BMI	8.87E-005	0.004	0.002	0.023	0.981
Gender	−0.010	0.095	−0.010	−0.107	0.915
ESS	−0.021	0.010	−0.250	−2.132	0.035
OSA severity	−0.003	0.051	−0.005	−0.057	0.954
FOSQ10	−0.009	0.007	−0.157	−1.267	0.208

a, dependent variable is CTES preference, R^2, 0.59. BMI, body mass index; ESS, Epworth Sleepiness Scale; OSA, obstructive sleep apnoea; FOSQ10, Functional Outcomes of Sleep Questionnaire with 10 questions.

try emerging treatments for OSA. Non-invasive electrical stimulation was the most preferred treatment compared with invasive electrical stimulation, and followed by CPAP and MADs. Less than one fifth of the respondents preferred to use CPAP, the current standard treatment for OSA. Less symptomatic patients, as demonstrated by a lower Epworth score, were more likely to choose a less invasive treatment option.

These results are important as new therapies for OSA are being developed. The biggest limitation of CPAP as a treatment for OSA is a relatively low long-term compliance, as demonstrated by McArdle *et al.*, who found that more than one-third of patients started on CPAP do not use it at all after 5 years (16). The benefits of controlled OSA include a reduction in daytime sleepiness and, in addition, less complications due to associated co-morbidities, such as hypertension, ischaemic heart disease, and stroke. These benefits are not seen, however, if patients cannot tolerate and comply with long-term treatment.

It is crucial, therefore, that alternative treatments to CPAP therapy are developed and tested. While these therapies are being developed, we need to take patients' preferences into consideration in order to achieve a good long-term compliance to OSA treatment, as it is likely that new treatments will not provide cure from the condition.

There are limitations to this study, however, with a limited dataset of only 162 patients filling out the survey.

This was a pre-selected cohort of mainly patients on CPAP, and the results require cautious interpretation due to a potential reporting bias. Nonetheless, the survey can be used as a guidance of public and patient involvement for future research interests aimed at developing alternative treatments for OSA.

Conclusions

Compliance remains the most significant limitation of CPAP as a long-term treatment for OSA. Due to significant short- and long-term complications of untreated OSA, the development of alternative treatments to CPAP is crucial to guarantee patients' adherence. The majority of patients that we tested preferred the potential use of emerging technologies and would potentially be available to test these methods in clinical trials. The preferred option was non-invasive electrical stimulation (CTES), followed by the invasive electrical stimulation (HNS), CPAP, and MADs. These findings are important in that they can be used to support future research applications into non-CPAP treatment methods for OSA.

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Respiratory sleep disorders in patients with congestive heart failure

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Abstract: Respiratory sleep disorders (RSD) occur in about 40-50% of patients with symptomatic congestive heart failure (CHF). Obstructive sleep apnea (OSA) is considered a cause of CHF, whereas central sleep apnea (CSA) is considered a response to heart failure, perhaps even compensatory. In the setting of heart failure, continuous positive airway pressure (CPAP) has a definite role in treating OSA with improvements in cardiac parameters expected. However in CSA, CPAP is an adjunctive therapy to other standard therapies directed towards the heart failure (pharmacological, device and surgical options). Whether adaptive servo controlled ventilatory support, a variant of CPAP, is beneficial is yet to be proven. Supplemental oxygen therapy should be used with caution in heart failure, in particular, by avoiding hyperoxia as indicated by SpO_2 values >95%.

Keywords: Sleep apnea; congestive heart failure (CHF)

Introduction

Sleep has a crucial role in defining the pattern of ventilation in patients with congestive heart failure (CHF). This is due to several factors (see *Table 1*). First, ventilation is under chemical control (arterial CO_2 levels) during sleep, where small changes such as 1-2 mmHg can alter respiratory drive enormously and lead to conditions such as central sleep apnea (CSA). In contrast during wakefulness, ventilation is under dual control—cortical and chemical. Cortical control allows speech, swallowing, laughter etc. to synchronize with ventilation.

Second, sleep is characterized by a reduction in skeletal muscle tone which results in loss of postural tone and need to sleep horizontally, but also reduces the respiratory pump muscle activity. The loss of upper airway muscle tone can potentially narrow the upper airway where there is an absence of bone, cartilage or other stabilizing structure causing snoring and/or obstructive sleep apnea (OSA). This will be more common in patients with small airways, such as those with "Asian" craniofacial appearance (1). Moreover,

sleep is associated with a change in body position (standing to supine) which has an effect of reducing lung volume.

Finally, sleep is associated with changes to the autonomic nervous system (reduction in sympathetic, rise in parasympathetic activity) and a ~25% reduction in cardiac output and minute volume of ventilation. Each of these factors underpins the potential development of respiratory sleep disorders (RSD) in CHF. RSD occur in ~50% of patients with CHF, equally spread between obstructive and central apnea in type (2). Unfortunately, most cases of apnea are unrecognized (3).

Obstructive sleep apnea (OSA)

Loss of upper airway muscle tone during sleep results in OSA. This condition is characterized by an imbalance between maintaining an open upper airway and the efforts of the respiratory pump musculature. When the upper airway collapses, the respiratory pump (intercostals and diaphragm) vigorously contract yet are unable to create airflow. This can result in significant hypoxemia and small

Table 1 Sleep related factors that contribute to RSD in CHF
Loss of skeletal muscle tone
Reduced minute volume of ventilation
Upper airway instability
Change from cortical to chemical (CO_2) control of ventilation
Change in posture (standing to supine) and effects on lung volume
Increased vagal and loss of sympathetic nervous control
Reduced heart rate & stroke volume
Reduced minute volume of ventilation
RSD, respiratory sleep disorders; CHF, congestive heart failure.

rise in CO_2 associated with large powerful negative, yet futile, intrathoracic pleural pressure swings (4) terminated usually 10-60 s later by a rapid change in sleep state, from deep to light sleep, associated with an arousal and restoration of airflow. Huge swings in systemic blood pressure occur plus release of inflammatory proteins and antioxidants which are thought to cause vascular damage. Localized vibrational damage to the carotid endothelium due to the energy produced during snoring, may also lead to atherosclerosis (5).

Central sleep apnea (CSA)

RSD caused by abnormal CO_2 control of ventilation can be divided into those with either a reduced ventilatory response to CO_2 or an increased ventilatory response to CO_2. The clinical scenario of a reduced response is characterized by hypoventilation associated with hypercapnia: this is seen in conditions such as chest wall disease (e.g., kyphoscoliosis), neurological diseases (e.g., motor neurone disease), drug induced (e.g., excessive sedatives) or mechanical factors (e.g., obesity hypoventilation syndrome).

RSD due to an increased ventilatory response to CO_2, leading to hypocapnia, is a little more complex and involve usually at least two of three physiological abnormalities: (I) prolonged lung to brain circulatory delay; (II) reduced lung capacity to prevent changes to PaO_2 and $PaCO_2$ and (III) an increased ventilatory response to CO_2. The prolonged lung to brain circulatory delay is commonly seen in CHF due to reduced cardiac output. In CHF, the circulatory delay is doubled (10-20 s). Reduced lung capacity to buffer swings in PaO_2 and $PaCO_2$ levels can be impaired if the lungs are small (restricted) or have impaired gas exchange properties (thickened basement membrane).

The heightened ventilatory response to CO_2 can involve the central (brainstem) or peripheral (carotid body) chemoreceptors (6). RSD associated with heightened ventilatory response to CO_2 are commonly seen in CHF, where it is known as CSA with a Cheyne Stokes pattern of Respiration (CSA-CSR). $PaCO_2$ levels oscillate above and below the apneic threshold thereby causing the typical hyperventilation and central apnea pattern. Hyperventilation and hypocapnia are common in such patients and is a useful guide to identify CSA-CSR (7). RSD of a similar pattern are seen in non-CHF conditions, such as periodic breathing at high altitude in normal subjects, premature infancy, and continuous positive airway pressure (CPAP) emergent CSA and narcotic ingestion.

Congestive heart failure (CHF)

CHF is defined as a syndrome of inadequate cardiac output to meet requirements, which is associated with symptoms (dyspnea, fatigue) and an objective cardiac abnormality. It can be caused by disorders of the left ventricular pump (commonly classified as either systolic *vs.* diastolic failure or idiopathic *vs.* ischaemic), arrhythmias (bradycardia, sick sinus syndrome, atrial fibrillation, heart block) and valvular disorders (8). Less common disorders such as pericardial diseases and myocardial infiltrative (e.g., amyloid) also need to be considered.

Understanding the cause of CHF is important when dealing with RSD, as there are bidirectional effects between CHF and RSD. Moreover some therapies common to sleep physicians (e.g., CPAP) may be effective in treating some causes of CHF (e.g., dilated cardiomyopathy) but not in others (e.g., aortic stenosis, mitral valve prolapse) (see *Figure 1*). In some cases, the CHF contributes to RSD, whereas in other RSD contribute to CHF. *Table 2* provides clinical indicators of OSA and CSA in patients with CHF.

Respiratory sleep disorders (RSD) contributing to CHF

OSA is the main RSD of concern in relation to cause of CHF. Research has provided the greatest understanding of its downstream cardiovascular effects.

Development of systemic hypertension as a precursor to CHF has been attributed to impaired baroreceptor control, impaired release of vasodilator endothelial nitric oxide and a generalized increase in sympathetic activity (9). Initially patients with OSA lose the usual nocturnal dip in systemic blood pressure and later they develop awaked
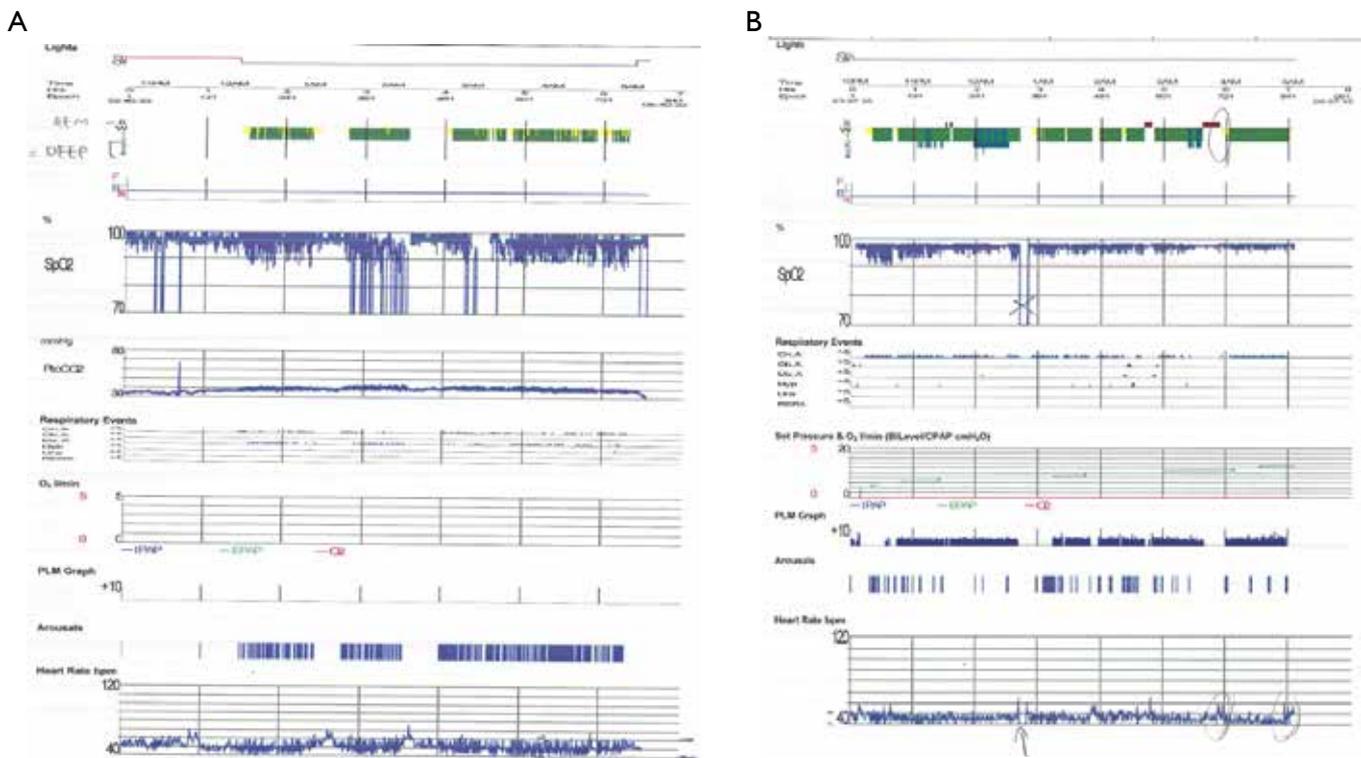
Figure 1 Overnight diagnostic and CPAP implementation polysomnograms in a 71-year-old male with mitral valve prolapse, atrial fibrillation and normal systolic function. The diagnostic study (A) indicates modest central sleep apnoea (AHI, 46 eph; minimum SpO_2, 75%) whilst 72 kg. Soon thereafter a CPAP study was performed in which oxygenation is much improved (minimum SpO_2, 90%), yet AHI is still high (32 eph) due to persistent central apnoeas. He later proceeded to a mitral valve repair and ablation of cardiac accessory conducting pathways with pacemaker insertion. Follow-up polysomnogram (B) without CPAP in sinus rhythm was then normal (AHI <5 events per hour and minimum SpO_2, 92%). This case highlights the difficulty in managing CSA-CSR with CPAP compared with the ease following mitral valve repair and abolition of atrial fibrillation. CPAP, continuous positive airway pressure; AHI, apnea hypopnea index; CSA-CSR, central sleep apnoea with cheyne stokes respiration.

hypertension and thereafter drug resistant hypertension (defined as needing at least three antihypertensive drug therapies). Thickening of the vascular media wall in the carotid arteries, whilst sparing the femoral arteries, has raised the possibility of snoring and vibrational damage to the endothelium (5).

Episodic atrial fibrillation is also common in patients with untreated OSA possibly related to surges in sympathetic activity whilst hypoxia and hypercapnia are present and the atrial walls are stretched. Atrial fibrillation is associated with ~25% reduction in cardiac output.

Coronary artery disease is not only more common in OSA patients, but it may be detected during polysomnography with ST depression on electrocardiograph (ECG). In patients with an established myocardial infarction, the relative risk of OSA is equivalent to a history of cigarette

smoking (10).

Stroke also occurs more commonly in OSA patients although the responsible mechanisms are not well understood. Theories include paradoxical emboli via transient opening of shunts (e.g., Patent Foramen Ovale), extreme swings in blood pressure beyond the levels that cerebral autoregulation can cope with, atrial fibrillation and atheromatous plaque rupture due to large intravascular and extravascular pressure swings.

CHF induced by OSA has also been observed based upon animal experiments (11), cross sectional and prospective epidemiological studies and human interventional studies. A toxic combination of repetitive hypoxemia, increases in afterload (negative intrathoracic pressures and increased systemic blood pressure) and recurring arousals are thought to be contributory.

Table 2 Clinical features that favour normal ventilation *vs.* OSA *vs.* CSA-CSR in heart failure

Normal ventilation during sleep

  Either gender

  Normal BMI and upper airway appearance

  Able to sleep flat in bed comfortably

  Absence of snoring

  Absence of apneas

  Absence of daytime fatigue or sleepiness

  Absence of hospital admissions for acute pulmonary edema

  Normal PaCO$_2$

Obstructive sleep apnea (OSA)

  Male predominance

  Elevated BMI or abnormally small upper airway

  Regular snorer

  Witnessed obstructive apneas

  Excessive daytime sleepiness

  Systemic hypertension on treatment

  Diastolic and possibly moderately severe left ventricular systolic failure

  Normal to high PaCO$_2$

Central sleep apnoea with Cheyne stokes respiration (CSA-CSR)

  Male predominance

  Normal BMI with normal upper airway

  Orthopnea and paroxysmal nocturnal dyspnoea

  May snore at peak of hyperventilation

  Witnessed central apnoeas

  Advanced heart failure of any cause

  Recurring episodes of pulmonary oedema

  Normal to low PaCO$_2$

  Abates in severity with more intense CHF treatment

  Periodic breathing on cardiopulmonary exercise testing

OSA, obstructive sleep apnea; CSA-CSR, central sleep apnoea with cheyne stokes respiration; CHF, congestive heart failure.

The evidence in favor of treating patients with both CHF and OSA is based primarily upon two randomized controlled trials (12,13): which in combination highlight improvement in objective markers and symptoms of cardiac function, whilst improvements in mortality are yet to be determined (*Table 3*). The SAVE trial (NCT00738179) of long term treatment of OSA with CPAP in high

cardiovascular risk patients may provide the answer (14).

CHF contributing to RSD

CHF may result in fluid retention and deranged autonomic control. Commonly, patients with CHF complain of dyspnea on exertion, then at rest or during sleep, where it is known as orthopnea or paroxysmal nocturnal dyspnea. Fluid retention results in interstitial edema, pleural effusion and alveolar edema. There may also be pedal edema and at times gut wall edema may lead to malabsorption.

Deranged autonomic control (heightened sympathetic and diminished vagal tone) is thought initially to be compensatory for the low cardiac output. This has an effect upon sleep quality [reduced sleep efficiency, paucity of slow wave and REM sleep, periodic limb movements] in addition to the development of tachyarrhythmias (atrial fibrillation, ventricular tachycardia, and ventricular fibrillation). Poor sleep quality can lead to daytime "fatigue", rather than true "sleepiness" (15).

Fluid retention can contribute to RSD via several mechanisms. First edema of the upper airway can narrow the lumen (and increase nasal resistance), and possibly the surface tension of the upper airway lining thus predisposing to upper airway collapse (snoring and obstructive apneas and hypopneas).

Second, shifting of fluid from lower limbs and abdomen to the thorax can contribute to transient pulmonary edema. This phenomenon is called "rostral fluid shift" (16) and if concentrated to the lungs cause CSA-CSR, or to the upper airway, OSA. Given that 50% of the oxygen stores are kept within the lungs, a reduction in size (total lung capacity) or function (altered gas exchange due to interstitial edema) can exacerbate periodic breathing (17).

Classically, lung function tests of patients with recurring episodes of acute heart failure have a restrictive ventilatory defect with a reduced diffusing capacity. In more extreme cases (e.g., mitral stenosis in youth) calcified pulmonary nodules and interstitial pulmonary fibrosis may be seen and represent the most severe form of pulmonary damage due to recurring pulmonary edema (17).

Third, treatments directed at fluid retention (e.g., diuretics) can alter upper airway surfactant properties (dry mouth) predisposing to upper airway collapse. Also, diuretic induced metabolic alkalosis can occasionally cause a compensatory hypoventilation and respiratory acidosis.

Deranged autonomic nervous system is most likely due to underlying CHF with a secondary RSD (18). Increased

Table 3 Comparison of the two major randomised controlled trials of CPAP for OSA in CHF due to left ventricular systolic failure

Comparison items	Kaneko (13)	Mansfield (12)
Patient number (completed/enrolled)	24/138	55/156
Drop-outs	Not stated	15/55
Study duration (months)	1	3
Threshold		
OSA/OSA & CSA (%)	>50	>80
AHI (eph)	>20	>5
LVEF (%)	<45	<55
CPAP (mean pressure × nightly use)	8.9×6.2	8.8×5.6
Outcomes		
Change in LVEF (%)	27-36	35-40
Left ventricular dimensions	Reduced	Not measured
Quality of life	Not measured	Improved
Blood pressure	Reduced	No change
Urinary norepinephrine	No measured	Reduced

CPAP, continuous positive airway pressure; OSA, obstructive sleep apnea; CSA, central sleep apnea; AHI, apnea hypopnea index; LVEF, left ventricular ejection fraction.

sympathetic activity causes hyperventilation, which underpins CSA-CSR. Whether the heightened sympathetic activity is caused by CSA-CSR or simply the underlying CHF is a point of debate. In the one study to address the issue (18), the severity of heart failure was a greater contributor to sympathetic activity than was the severity of CSA-CSR.

The increased ventilatory response to CO_2, coupled with prolonged lung to brain circulatory delay (low cardiac output) and abnormal lung function (pulmonary edema) leads to a cyclic breathing pattern (19). Thirty seconds periods of hyperventilation are interspersed with 20-30 s periods of central apnea in a crescendo decrescendo pattern. CSA is often triggered by an arousal or sudden state change. An arousal occurs at the peak of ventilation. Often there is an absence of hypoxemia. Concurrent periodic limb movements are common. Usually CSA-CSR is worse with transition from wake to stages 1 and 2 non-REM sleep and less so in slow wave and REM sleeps. Of interest, as with OSA, it is more common in the supine position, less in the lateral position (20) and diminished further when the head of the bed is raised (21).

If one measure heart rate and blood pressure continuously in patients with CSA-CSR, one will see that the cardiorespiratory pattern has a cycle length of about 60 s with rise of ventilation associated with a rise in blood pressure and heart rate (*Figure 2*). Males appear to be over represented in the CSA-CSR populations for reasons

that are unclear: perhaps related to a greater respiratory muscle mass and the ability to increase minute volume of ventilation sufficiently to drive the CO_2 level below the apneic threshold.

An important distinction with CSA-CSR is the cycle length, which includes the apnea and the hyperventilation (hyperpnoea) component (*Figure 3*) (22,23). Two aspects need highlighting. The first is that the ratio of the hyperpnoea to apnea is usually >1 in CHF. Moreover the duration of the cycle length is 45-75 s in CHF (*Figure 3A*). In contrast, non CHF causes of CSA have a hyperpnoea to apnea ration of <1 and a cycle length 20-40 s (*Figure 3B*). This ratio can assist in understanding loop gain, a descriptive term for periodic breathing (24,25). The cause of non-CHF CSA, such as that due to narcotic ingestion, or unknown causes (so called "idiopathic") can be distinguished from CHF induced CSA-CSR by the pattern of ventilation seen on the polysomnogram. Similar short cycle length CSA can be seen with CPAP initiation (which usually resolves over 1-3 months duration), at high altitude in normal and premature infants.

Is CSA-CSR detrimental?

Whether CSA-CSR contributes to the development of CHF has not been proven (26). Some evidence exists that

Figure 2 This 5-min polysomnogram montage showing classic cyclic central sleep apnoea with accompanying cyclic rise and fall in heart rate. Note minimal hypoxemia and cycle length ~50 s.

the periodic hyperventilation indeed assists cardiac output (27,28) and that the hyperventilation results in an increase in end-expiratory lung volume (29) thereby offsetting the restrictive ventilatory defect and creating a respiratory alkalosis which may prevent the development of hypercapnic acidosis. Acidosis has a far worse effect on the heart's contractility in the setting of hypoxemia than does alkalosis (26). Recent unpublished research from our laboratory, where we compared minute volume of ventilation and intrathoracic pressure swings during sleep in CSA-CSR and OSA indicated that both apnea types have similar degrees of work of breathing (pressure × volume), however CSA-CSR does so with greater efficiency (pressure × time).

Instead, CSA-CSR is a marker of advanced heart failure. Elevated pulmonary capillary wedge pressure (30), BNP and noradrenaline levels (31,32), low left ventricular ejection fraction (LVEF) and other markers of unstable or advanced CHF have been shown to occur with CSA-CSR. Thus when recognized, it should trigger attention to determine whether more aggressive therapy is required to combat the CHF. This might include sleeping upright (19), or laterally (20), greater afterload reduction (vasodilators), preload reduction [diuretics such as acetazolamide (33)],

inotropes, surgery (coronary artery graft surgery, valvular repair or replacement), rhythm correction, pacemakers [atrial overdrive (34), biventricular (35)], left ventricular assist device (36) or even heart transplantation (37).

Continuous positive airway pressure (CPAP)

Included in the regime of more aggressive therapy towards CHF is CPAP. Although commonly used for OSA, the mechanisms of action in CHF with CSA-CSR differ and may be limited to a subset of patients in whom the cause of CHF is amenable to CPAP (such as a dilated cardiomyopathy, but not aortic stenosis (*Figure 1*). The actions of CPAP can be divided into (I) upper airway pneumatic dilating; (II) pulmonary and (III) cardiac (*Table 4*).

The pulmonary effects of CPAP include increasing lung volume, preventing alveolar collapse and improving oxygen storage and gas transfer and bronchodilation (38). Assistance with respiratory muscles and abolition of negative intrathoracic pressure has an estimated 40% reduction in the work of breathing (39), which will minimize the cardiac output required by the respiratory muscles. Thus, CPAP may reduce the cardiac output "steal" phenomenon by the

Figure 3 These two 4-min polysomnogram montages indicate (A) CHF pattern with CSA-CSR and a long apnoea-hyperpnoea cycle length pattern (B) non-CHF (i.e., idiopathic) pattern of CSA with short apnea hypopnea cycle length in patients with normal cardiac function. Cycle lengths greater than 45 s helps distinguish heart failure from non-heart failure patterns. CHF, congestive heart failure; CSA-CSR, central sleep apnoea with cheyne stokes respiration.

Table 4 Actions of continuous positive airway pressure in CHF

Scenario	Acute pulmonary oedema	OSA	CSA-CSR
Upper airway			
Pneumatic splinting	+	+++	+
Pulmonary			
Overcome negative ITP	+	+++	+
Increase lung volume	++	++	++
Prevent alveolar collapse	+++	+	++
Improve VQ matching	+++	+	++
Reduce pulmonary vasoconstriction	+++	+	++
Assist inspiratory muscles	+++	+	++
Cardiac			
Afterload reduction			
Less negative ITP	+	+++	+
Fall in systemic BP	+	++	++
Reduced LV diameter	+	++	++
Preload reduction	++	+	+

Three common scenarios: +, a slight effect; ++, a moderate effect; +++, a major effect. CHF, congestive heart failure; OSA, obstructive sleep apnea; CSA-CSR, central sleep apnoea with cheyne stokes respiration; ITP, intrathoracic pressure; VQ, ventilation perfusion.

overworked respiratory muscles during sleep (40). It is also possible that CPAP induces improved ventilation perfusion (VQ) matching, reduces acute pulmonary hypertension and thereby prevents intermittent shunting through patent foramen ovale (or similar). In addition CPAP may also provide a small but significant rise in dead space, thus increasing CO_2 levels. Importantly minute ventilation falls

and $PaCO_2$ levels rise in patients with CSA-CSR treated with CPAP therapy.

The cardiac effects of CPAP include reductions in left ventricular afterload (38) and preload. The afterload falls because the negative intrathoracic pressure is replaced by positive pressure, upstream blood pressure falls (less sympathetic activity) and the cardiac chamber size is

Figure 4 High resolution CT scan of the chest indicating absence of emphysema or interstitial lung disease despite a reduced TLCO.

reduced (41). Reductions in cardiac sympathetic activity (42) and myocardial oxygen demand have been reported immediately (43) and the long term (44). Preload may also fall by reducing venous return to the thorax.

Thus the effects of CPAP are complex and are likely to differ in the following settings: OSA, CSA-CSR and acute cardiogenic pulmonary edema (*Table 4*). Also some CHF may not be amenable to CPAP particularly if dry or CHF due to some valvular or rhythm disturbances (45).

The main trial conducted to date of 2-years of CPAP treatment in 258 CHF patients with CSA-CSR (CANPAP trial) did show improvements in apnea hyponea index (AHI), oxygenation, 6 minutes-walk distance, LVEF and plasma norepinephrine, however there was no overall difference in hospitalizations, quality of life or transplant free survival (46). A post hoc analysis, dividing those patients who had a fall in AHI to <15 at 3 months with CPAP did have a survival benefit (47).

Whether adaptive servo controlled ventilation (ASV) support is better than CPAP has not been proven (47). ASV provides CPAP with variable ventilatory support which increases during the central apneas (48). Two large clinical trials comparing ASV with best practice (NCT01128816 & NCT00733343) are underway. The preliminary results of one study with 1325 subjects followed for over 2 years in 267 study locations, the SERVE-HF study indicated a 2.5% absolute increased annual risk of cardiovascular mortality for those randomised to ASV therapy compared with the control group (49). However the primary endpoint (all-cause mortality or unplanned hospitalization for worsening heart failure) was not significantly different.

Reasons for the increased cardiovascular mortality with ASV are unclear; possibilities include inadequate preload, which is necessary for positive airway pressure therapy.

Oxygen therapy

Oxygen therapy is often considered in patients with CHF. Correction of hypoxemia (SpO_2 <90%) is important and can be achieved usually with CPAP or supplemental oxygen therapy. An optimal SpO_2 value for patients with CHF is 92-95%. However, most patients with CHF are not hypoxic and if they are (such as during acute pulmonary edema), the oxygen levels promptly rise to SpO_2 values >92% with CPAP (at a level of approximately 10% of body weight, i.e., 70 kg body weight =7 cmH_2O CPAP). Moreover, supplemental oxygen therapy has not been shown to improve cardiac function in any clinical trial. Indeed the converse is true. Two studies of oxygen therapy in CHF have shown the cardiac output to fall and pulmonary capillary wedge pressure to rise (50,51). In addition, supplemental oxygen has not been shown to be of benefit in myocardial infarction. Complications of excessive supplemental oxygen use are starting to emerge beyond the well-recognized adverse effects of hypercapnia and pulmonary fibrosis. Thus supplemental oxygen is not routinely recommended in normoxic CHF patients (52).

Case study

A 68-year-old male, previous smoker developed a myocardial infarct in 2004, then atrial fibrillation and pulmonary edema in 2005 requiring a pacemaker. Associated iron deficient anemia related to peptic ulcer was noted. He had progressive dyspnea needing hospitalization in 2014 for heart transplant assessment. There was a history of snoring and witnessed apneas extending for more than 10 years. New York Heart Association class 3. His Epworth sleepiness score was 7 (normal <10, abnormal 10-24).

Examination revealed BMI 24 and crowded oropharynx (Mallampati 3). LVEF by nuclear gated scan was 15%, and a right heart catheter study revealed an elevated pulmonary capillary wedge pressure of 21 mmHg. Lung function tests indicated normal spirometry and static lung volumes, yet reduced TLCO at 62% predicted. High resolution scan did not identify emphysema (*Figure 4*). Cardiopulmonary exercise test indicated a heightened VE to VO_2 slope, an absence of hypoxemia and reduced maximal oxygen consumption (VO_2 peak) of 12.6 mL/min/kg (*Figure 5*).

Predicted | Measured (% pred.)

VEmax (L/min) 127 46 (36%)
HRmax (bpm) 153 118 (77%)
Wmax (W) 172 80 (47%)
VO₂peak (L/min) 2.81 1.06 (38%)
VO₂peak (mL/min/kg) 12.6
Exercise time: 8 mins (Winc=10 watts)

Borg symptoms
Dysponea: 4
Leg fatigue: 3
Chest pain: 0

Resting BP: 85/50   Peak BP: 100/60
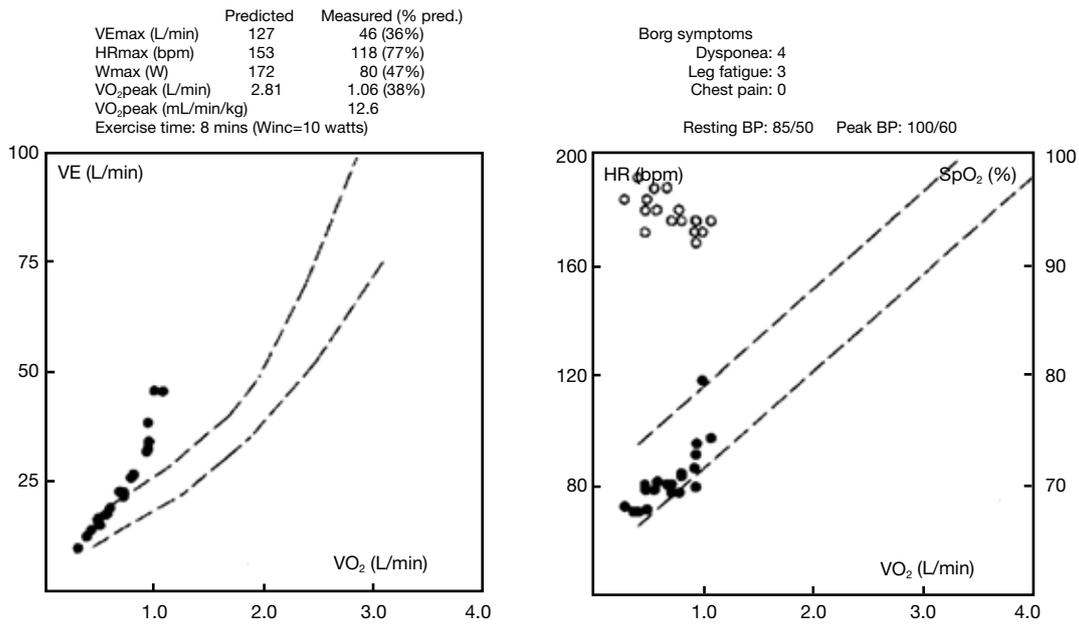
Figure 5 Graphic display of cardiopulmonary exercise test. Note elevated VE/VO₂ slope (indicative of hyperventilation) and absence of hypoxemia with exercise.
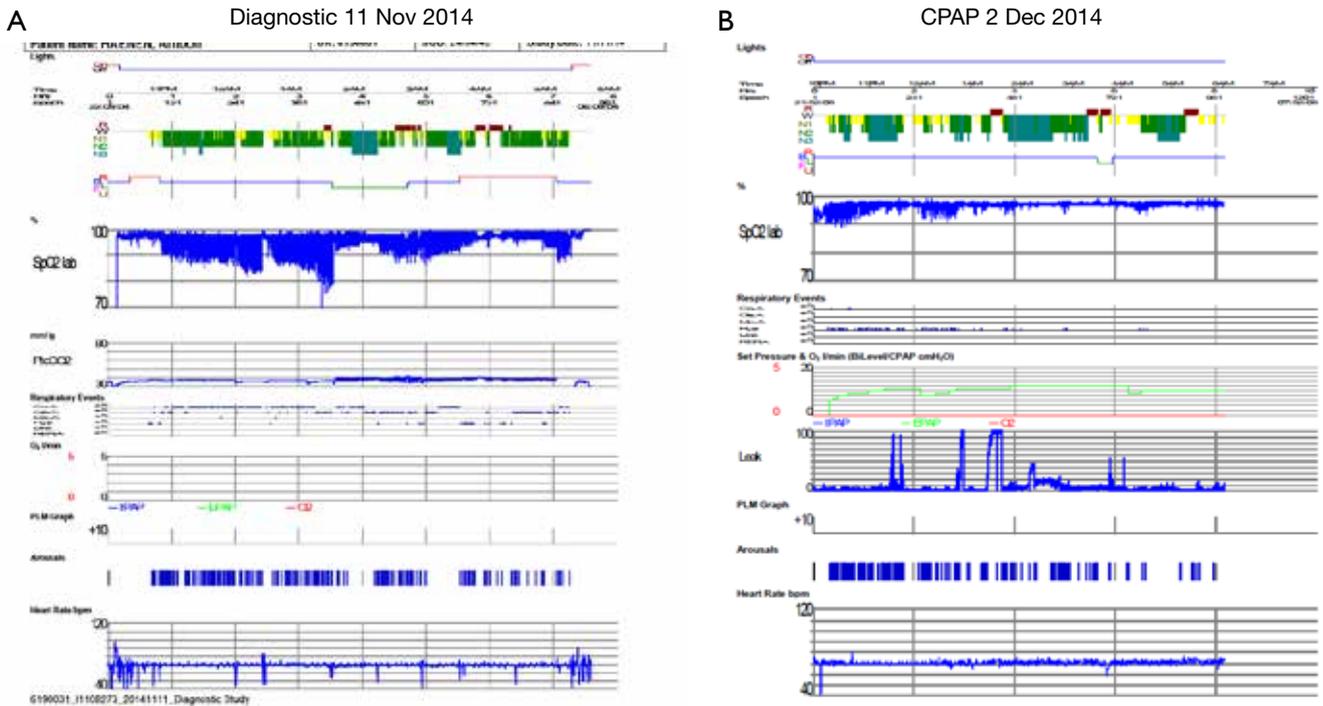
Figure 6 Overnight diagnostic and CPAP implementation polysomnograms: diagnostic (A) side and CPAP titration on (B) in 68-year-old male with ischemic cardiomyopathy (note improved oxygenation with CPAP). CPAP, continuous positive airway pressure.
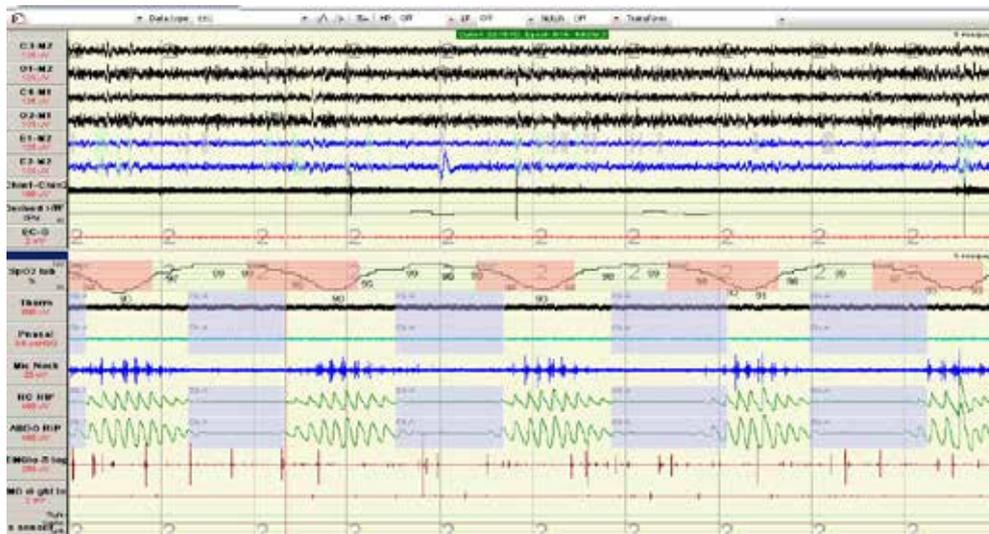
Figure 7 A 5-min diagnostic polysomnogram montage illustrating classic long cycle length CSA-CSR, snoring at peak of ventilation and absence of hypoxemia. CSA-CSR, central sleep apnoea with cheyne stokes respiration.
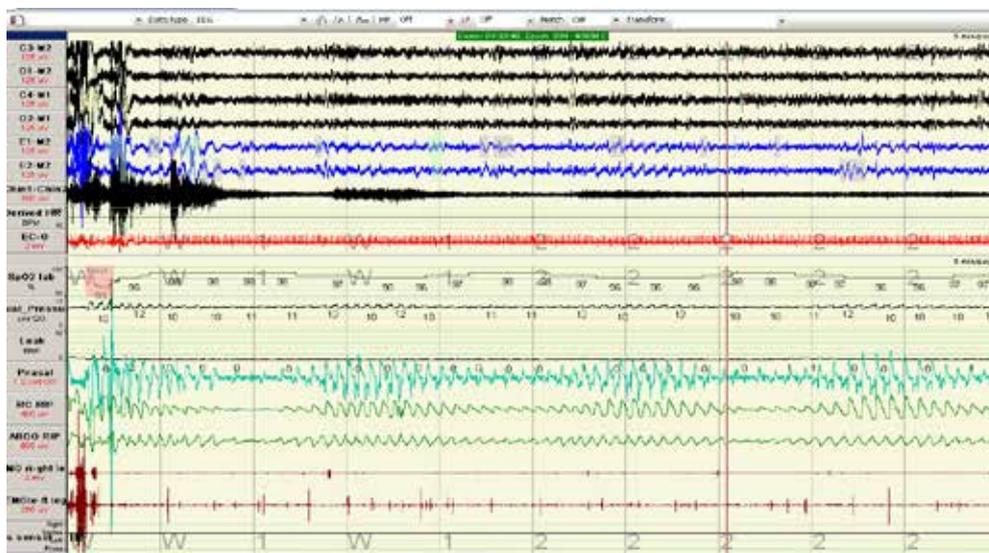
Figure 8 A 5-min CPAP implementation polysomnogram montage illustrating immediate response to CPAP with marked reduction of CSA-CSR. CPAP, continuous positive airway pressure; CSA-CSR, central sleep apnoea with cheyne stokes respiration.

Two overnight polysomnograms indicated severe sleep apnoea on the diagnostic night: central during non REM sleep and obstructive during REM sleep and PaCO$_2$ levels were 37 and 35 mmHg at the beginning and end of the diagnostic study. Transcutaneous PCO$_2$ levels were <37 mmHg across the night. There was a marked improvement during the CPAP study less than 4 weeks later (*Figures 6-8*). Note

there was no significant weight change (84-86 kg). With CPAP, there was an increase in supine position (56-96% of the night) and slow wave (11-28% of total sleep time) sleep. The AHI fell (50-16 events per hour) and minimum SpO$_2$ levels rose (70-89%). Significant objective and symptomatic improvements in heart failure followed.

This case highlights (I) the under recognition of

disorders of respiration during sleep in high risk patients (ischemic heart disease, atrial fibrillation); (II) the overlap of central and obstructive sleep apnoea within the same patient and (III) the immediate response to CPAP in patients with ischemic dilated cardiomyopathy.

Conclusions

Identification of RSD is important in patients with CHF. On one hand, identification and treatment of OSA may improve cardiac function both objectively and symptomatically. On the other hand, identification of CSA is indicative of advanced heart failure with subacute pulmonary edema and the need to augment therapy directed towards the underlying heart failure. This therapy may include pharmacological, surgical and device options (including CPAP). Oxygen therapy should not be used routinely, unless hypoxemic and unresponsive to standard CHF therapies mentioned above.

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None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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The pathogenesis of obstructive sleep apnea

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Abstract: Obstructive sleep apnea (OSA) is a major source of cardiovascular morbidity and mortality, and represents an increasing burden on health care resources. Understanding underlying pathogenic mechanisms of OSA will ultimately allow for the development of rational therapeutic strategies. In this article, we review current concepts about the pathogenesis of OSA. Specifically, we consider the evidence that the upper airway plays a primary role in OSA pathogenesis and provide a framework for modelling its biomechanical properties and propensity to collapse during sleep. Anatomical and neuromuscular factors that modulate upper airway obstruction are also discussed. Finally, we consider models of periodic breathing, and elaborate generalizable mechanisms by which upper airway obstruction destabilizes respiratory patterns during sleep. In our model, upper airway obstruction triggers a mismatch between ventilatory supply and demand. In this model, trade-offs between maintaining sleep stability or ventilation can account for a full range of OSA disease severity and expression. Recurrent arousals and transient increases in airway patency may restore ventilation between periods of sleep, while alterations in neuromuscular and arousal responses to upper airway obstruction may improve sleep stability at still suboptimal levels of ventilation.

Keywords: Obstructive sleep apnea (OSA); upper airway; inspiratory flow limitation; Starling resistor

Introduction

Obstructive sleep apnea (OSA) is a highly prevalent disease, characterized by upper airway collapse during sleep resulting in recurring arousals and desaturations. Estimates of disease prevalence range between 3% and 10% of the population (1,2). Prevalence has risen with escalating rates of obesity, a major risk factor for OSA (2,3). Significant clinical consequences of the disorder cover a wide spectrum and include daytime hypersomnolence, neurocognitive dysfunction, cardiovascular disease, metabolic dysfunction, respiratory failure, and cor pulmonale (4-16). As a result, OSA represents an increasing burden on health care resources. Understanding underlying pathogenic mechanisms of OSA will ultimately allow for the development of rational therapeutic strategies in an era of

personalized medicine.

In this article, we will review current concepts about the pathogenesis of OSA. Specifically, we will consider factors that initiate upper airway obstruction during sleep and examine responses to airway obstruction over the course of the ensuing event. In considering factors responsible for the initiation of an obstructive apnea, we will model the alterations in pharyngeal biomechanics that to lead airway obstruction during sleep. When the pharynx collapses, airflow obstruction elicits neuromuscular responses that can mitigate the obstruction and restore airway patency and ventilation. If these neuromuscular mechanisms are inadequate, additional factors contribute to the development of recurrent periods of airway obstruction and arousals from sleep. In this context, models that predict the likelihood of developing recurrent sleep disordered breathing episodes
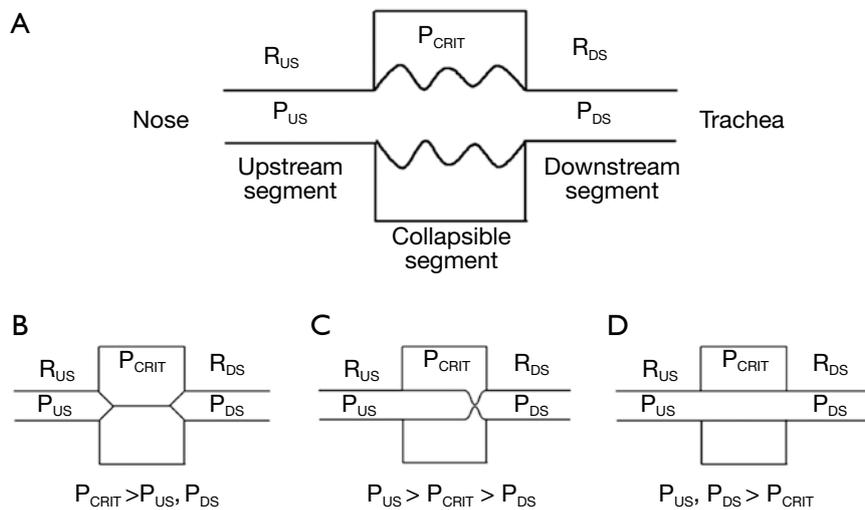
Figure 1 Starling resistor model of the upper airway: (A) A collapsible segment is bordered by rigid upstream (nasal passages) and downstream (trachea) segments. These rigid segments are characterized by intraluminal pressures (P_{US} and P_{DS} in the upstream and downstream segments, respectively) and resistance to airflow (R_{US} and R_{DS}, respectively); (B) when P_{US} and P_{DS} are less than P_{CRIT}, the airway is closed; (C) when P_{US} is greater than P_{CRIT}, but P_{DS} falls below P_{CRIT}, the airway becomes flow limited on inspiration and can rapidly cycle between an open and closed state; (D) when both P_{US} and P_{DS} remain above P_{CRIT}, the airway is patent. Adapted from Gleadhill *et al*. 1991 (25).

will be considered. In modelling recurrent sleep disordered breathing episodes, we will elaborate generalizable mechanisms by which upper airway obstruction destabilizes respiratory and sleep-wake patterns.

Upper airway biomechanics

OSA is characterized by recurrent periods of upper airway occlusion during sleep (17). In modeling the biomechanics of pharyngeal airflow obstruction, we consider the fact that the upper airway collapses dynamically during sleep and reopens during wakefulness. Investigators have previously modelled dynamic alterations in patency as a function of transmural pressure across collapsible segments in biologic conduits in the cardiovascular, gastrointestinal, and genitourinary systems (18-24) (*Figure 1*). In the case of the upper airway, the collapsible segment is bordered by two rigid segments upstream (nasal passages) and downstream (trachea) (*Figure 1A*) (26). The segments upstream and downstream to the collapsible site have fixed diameters and resistances, R_{US} and R_{DS}, respectively, and the pressures upstream and downstream are P_{US} and P_{DS}, respectively.

Several important features of this model, known as the Starling resistor, are worth emphasizing. When P_{US} and P_{DS} are less than the critical pressure surrounding the collapsible

segment (P_{CRIT}), the transmural pressure is negative, the airway closes and airflow ceases (*Figure 1B*). Flow can be re-established by raising P_{US} above P_{CRIT}. If both P_{US} and P_{DS} are greater than P_{CRIT}, however, transmural pressure remains positive (*Figure 1D*). Under these conditions, flow through the upper airway is proportional to the pressure gradient across the entire airway, and can be described by the voltage-current relationship in Ohm's law:

$$V_I = \frac{P_{US} - P_{DS}}{R_{US} + R_{DS}} \quad [1]$$

In contrast, when P_{US} is greater than P_{CRIT} and P_{DS} is less than P_{CRIT}, however, the airway enters a flow-limited condition (*Figure 1C*). To illustrate the mechanism by which inspiratory flow limitation arises, consider the effects of lowering P_{DS} progressively during inspiration. At the start of inspiration, flow commences upon lowering P_{DS}. As inspiration progresses and P_{DS} falls below P_{CRIT}, the airway begins to collapse. If the upper airway were to occlude, flow would cease transiently. As the upper airway occludes, the pressure immediately upstream of the occlusion would equilibrate with P_{US} and rise above P_{CRIT}. This increase in pressure would inevitably lead to reopening of the airway. As the airway cycles rapidly between an open and closed state, the pressure at the collapsible segment remains
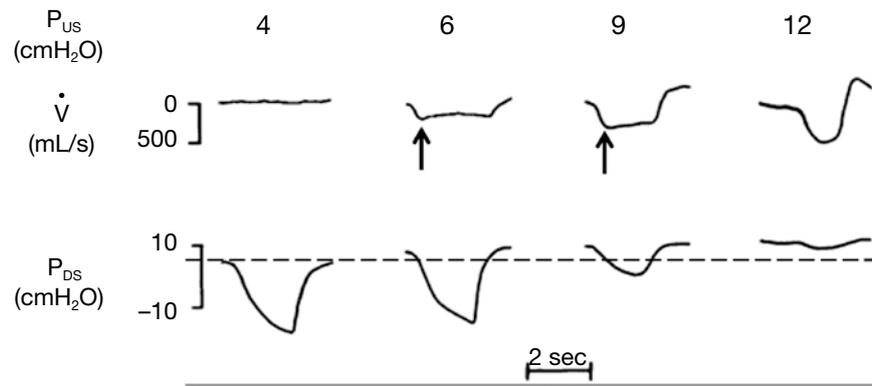
Figure 2 The effects of varying the upstream nasal pressure on inspiratory flow. At P_{US} of 4 cmH$_2$O, P_{US} is less than the critical closing pressure, P_{CRIT} (dashed line). As a result, the upper airway is closed, and flow cannot occur. At P_{US} of 6 and 9 cmH$_2$O, P_{US} is greater than P_{CRIT}, and flow is established. As inspiration progresses, P_{DS} falls below P_{CRIT}, which causes flow to plateau at maximal inspiratory flow ($V_I$max, arrows), denoting the onset of flow limitation in mid-inspiration. At P_{US} of 12 cmH$_2$O, both P_{US} and P_{DS} remain above P_{CRIT} throughout inspiration, and flow limitation is abolished. Adapted from Schwartz *et al*. 1998 (27).

nearly constant at P_{CRIT}. Because pressure in the collapsible segment is constant, airflow also remains constant. Under these circumstances, flow becomes independent of P_{DS} and plateaus at a maximal level ($V_I$max) as P_{CRIT} replaces P_{DS} and becomes the effective back-pressure to inspiratory flow. The level of $V_I$max is therefore governed by the pressure gradient between P_{US} and P_{CRIT} and the resistance across the upstream segment, according to the following equation:

$$V_I \max = \frac{P_{US} - P_{CRIT}}{R_{US}} \quad [2]$$

In this model, decreasing P_{DS} does not cause the upper airway to occlude and cannot account for the development of obstructive apneas during sleep.

Now consider the effects of altering P_{US} on inspiratory flow in *Figure 2*. The effects of increasing levels of CPAP on upper airway pressure-flow dynamics is represented. In this figure, CPAP is applied at pressures 4, 6, 9 and 12 cmH$_2$O. At a low nasal pressure (4 cmH$_2$O), both P_{US} and P_{DS} are less than P_{CRIT}, the airway occludes and no flow occurs. At an intermediate pressure of 6 cmH$_2$O, P_{US} exceeds P_{CRIT} and flow is re-established. Nevertheless, P_{DS} still falls below P_{CRIT} over the course of inspiration, resulting in a plateau of mid-inspiratory airflow (flow-limitation) at $V_I$max (see arrows). With further increases in nasal pressure from 6 to 9 cmH$_2$O, inspiratory flow plateaus at a proportionately higher level, as described by Eq. [2], but flow still remains limited. Finally, at nasal pressure of 12 cmH$_2$O, both P_{US} and P_{DS} have increased sufficiently such that both pressures remain

above P_{CRIT} throughout inspiration and flow limitation is abolished. Conversely, decreases in P_{US} elicit flow limitation and progressive decreases in $V_I$max until flow ceases (the upper airway occludes) when P_{US} falls below P_{CRIT}. A simple pressure-flow plot (*Figure 3*) describes this linear relationship between $V_I$max and P_{US} (Eq. [2]). This relationship can be used to define P_{CRIT} at the zero flow intercept and R_{US} as the reciprocal of the slope of this line (27,28).

It is important to note that inspiratory airflow limitation exerts two distinct loads on the respiratory system. First, airway resistance increases markedly in the flow limited compared to the non-flow limited state. During non-flow limited breathing (in the absence of upper airway collapse), the combined resistances of the upstream and downstream segments (Eq. [1]) is approximately 1 to 2 cmH$_2$O/L/s, which accounts for approximately half of the total resistance of the respiratory system during tidal breathing. In contrast, resistance of the upstream segment alone (Eq. [2]) during periods of inspiratory airflow limitation increases into the range of 20 to 40 cmH$_2$O/L/s. Second, additional load is imposed on the respiratory system during periods of flow limitation by virtue of the fact that patients continue to exert ever-increasing effort without increasing inspiratory airflow. In essence, a large portion of the pressure generated by the respiratory pump muscles is wasted by dynamic collapse of the upper airway without augmenting ventilation. Thus, increases in airway resistance and dynamic collapse of the upper airway augment work of breathing during periods of inspiratory airflow limitation and/or complete upper airway obstruction.
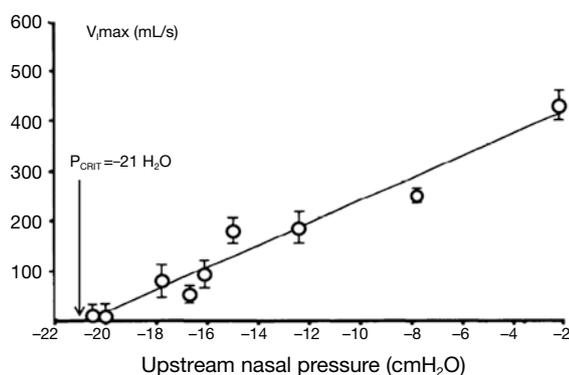
Figure 3 Relationship between maximal inspiratory flow ($V_I$max ± standard deviation) and upstream nasal pressure (P_{US}) during NREM sleep. Inspiratory flow limitation in a non-apneic normal individual was induced by sequentially lowering P_{US}. Under these conditions, $V_I$max is linearly dependent on P_{US}. The reciprocal of the line slope represents the upstream resistance (R_{US}). The critical pressure (P_{CRIT}) is the zero-flow intercept, –21 cmH$_2$O (indicated by arrow), indicating total upper airway occlusion. Adapted from Schwartz *et al*. 1988 (27).

The role of upper airway obstruction in OSA pathogenesis

Current evidence suggests that disturbances in P_{CRIT} play a primary role in OSA pathogenesis. The role of pharyngeal obstruction in OSA pathogenesis can be considered in light of Koch's postulates (29), which establish criteria for demonstrating a causal relationship between pathogenic factors that promote upper airway obstruction and the overt polysomnographic manifestation of the disease. These principles require first and foremost that pathogenic factors causing upper airway collapse are associated with OSA. Investigators have examined the association between pharyngeal collapsibility and the clinical manifestations of OSA in several observational studies (25,28,30-35). Elevations in P_{CRIT} have been demonstrated in OSA patients compared to age, sex and body mass index (BMI) matched controls under general anesthesia and neuromuscular blockade (35) as well as during sleep (25).

Further strength for the association between upper airway obstruction and OSA pathogenesis is evidenced by studies demonstrating that pharyngeal collapsibility (P_{CRIT}) is both a sensitive (a large proportion of persons with OSA have collapsible upper airways) and specific (a large proportion of normal persons do not have
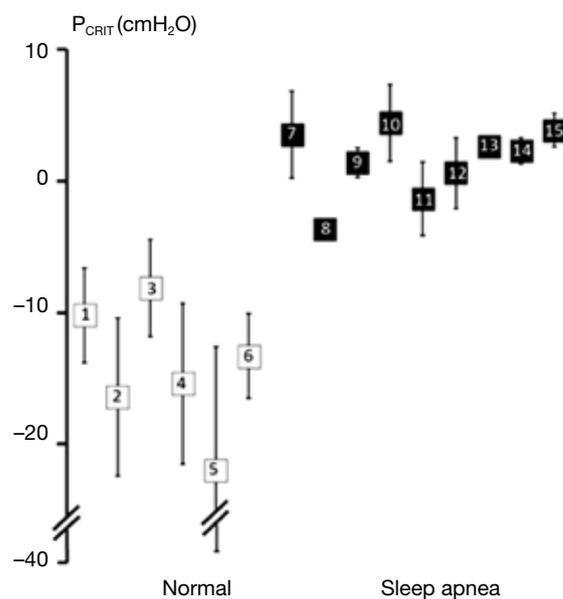
Figure 4 Summary of studies examining upper airway collapsibility (critical closing pressure, P_{CRIT}) in normal individuals and obstructive sleep apnea (OSA) patients. Mean P_{CRIT} (± standard deviation) in non-apneic controls (open squares) and apneic subjects (closed squares) are plotted. P_{CRIT} is elevated in sleep apnea patients compared to normal controls. Furthermore, these studies demonstrate minimal overlap of P_{CRIT} between disease and health, suggesting that an elevated P_{CRIT} is both sensitive and specific for OSA. Source studies, as indicated by enclosed numbers, are: 1. Meurice *et al*., 1996 (37); 2. Meurice *et al*., 1996 (36); 3. Issa & Sullivan, 1984 (30); 4. Gold *et al*., 2002 (33); 5. Philip-Joet *et al*., 1996 (38); 6. Schwartz *et al*., 1988 (27); 7. Smith *et al*., 1988 (28); 8. Ng *et al*., 2003 (39); 9. Kirkness *et al*., 2003 (34); 10. Farre *et al*., 2003 (40); 11. Series *et al*., 1996 (42); 12. Gold *et al*., 2002 (33); 13. Sforza *et al*., 2000 (32); 14. Sforza *et al*., 1999 (31); 15. Issa & Sullivan, 1984 (30). Adapted from Pack, 2009 (41).

collapsible airways) finding in OSA. High levels of sensitivity and specificity of P_{CRIT} can be inferred from numerous studies that have demonstrated quantitative differences in P_{CRIT} between health and disease (27,28,30-34, 36-41). In the aggregate, these studies demonstrated that nearly all persons with OSA have a P_{CRIT} greater than –5 cmH$_2$O, indicating that upper airway collapsibility is a sensitive marker for OSA (*Figure 4*). In contrast, elevations in P_{CRIT} were nearly absent in normal controls, suggesting that increased pharyngeal collapsibility is also highly specific to OSA.
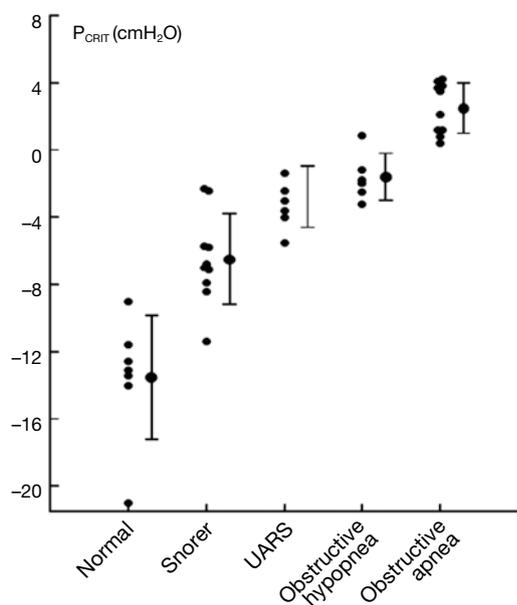
Figure 5 Relationship between P_{CRIT} and clinical disorders characterized by upper airway obstruction during sleep. As P_{CRIT} increases, the severity of upper airway obstruction also increases progressively, suggesting a dose-response relationship between upper airway collapsibility and worsening obstruction in asymptomatic snorers and patients with upper airway resistance syndrome (UARS), obstructive hypopneas, and obstructive apneas. Adapted from Schwartz *et al*. 1988 and Gleadhill *et al*. 1991 (25,27).

Additional evidence for the primacy of upper airway collapse in OSA pathogenesis is provided by studies demonstrating a dose-response relationship between pharyngeal collapsibility and severity of OSA (*Figure 5*). As P_{CRIT} rises progressively, increases in severity of upper airway obstruction during sleep have also been observed clinically. Modest elevations in P_{CRIT} have been associated with snoring, whereas moderate elevations in P_{CRIT} to levels between –5 and 0 cmH_2O have been associated with sleep disordered breathing characterized primarily by obstructive hypopneas. With further increases in P_{CRIT} (P_{CRIT} becomes positive), periodic obstructive apneas are observed during sleep. Quantitative differences in P_{CRIT} have therefore been associated with graded changes in the severity of airway obstruction during sleep.

Studies inducing experimental upper airway collapse during sleep also implicate pharyngeal obstruction in OSA pathogenesis. Indeed, manipulating nasal pressure recapitulates the entire OSA disease spectrum. With the

application of subatmospheric nasal pressure during sleep, stable flow limited breathing and snoring were observed in healthy test subjects (27). Further reductions in nasal pressure resulted in recurrent obstructive hypopneas and apneas, which occurred at a rate of ~20-40 episodes per hour and were associated with oxyhemoglobin desaturations and arousals. Continuous application of subatmospheric nasal pressure during sleep also caused alterations in sleep architecture, with increases in stage 1 and stage 2 sleep, and decreases in stage 3/4 and REM sleep compared to baseline (43). Moreover, when study participants were subjected to two consecutive nights of experimentally induced OSA, multiple daytime sleep latency times fell markedly, indicating that excessive daytime somnolence had developed. Thus, experimental evidence suggests that airway collapse alone is sufficient to cause OSA.

Conversely, OSA can be treated with interventions designed to restore upper airway patency, further fulfilling Koch's postulate that upper airway collapse is necessary for disease pathogenesis. In fact, treatments that decrease P_{CRIT} (e.g., weight loss or uvulopalatopharyngoplasty) lead to improvements in OSA and to resolution of disease when P_{CRIT} falls below –5 cmH_2O (27,44). At this level of P_{CRIT}, a transmural pressure of 5 cmH_2O provides adequate airflow to stabilize breathing patterns during sleep. Similarly, a positive transmural pressure can be induced by increasing P_{US}, leading to resolution of upper airway obstruction. With application of progressively increasing nasal pressure during CPAP titration, upper airway obstruction and recurrent obstructive apneas and hyponeas are reversed. As nasal pressure increases, episodic obstructive apneas give way to hypopneas when P_{US} rises above P_{CRIT}. Further increases in nasal pressure stabilize respiratory patterns, leading to regular snoring and ultimately to the resolution of flow-limitation altogether as CPAP pressures rise to therapeutic levels.

Finally, the relationship between upper airway collapsibility and OSA is biologically plausible, as exemplified by several approaches to modelling upper airway obstruction. First, airway obstruction could be due to increasing airway resistance that is produced by narrowing of an otherwise rigid structure. In this model, no matter how narrow the tube, flow still remains dependent on downstream pressure. Although flow is reduced when resistance is high, this model cannot account for the development of inspiratory flow limitation (or snoring) in which airflow becomes independent of downstream pressure. Second, investigators have postulated that
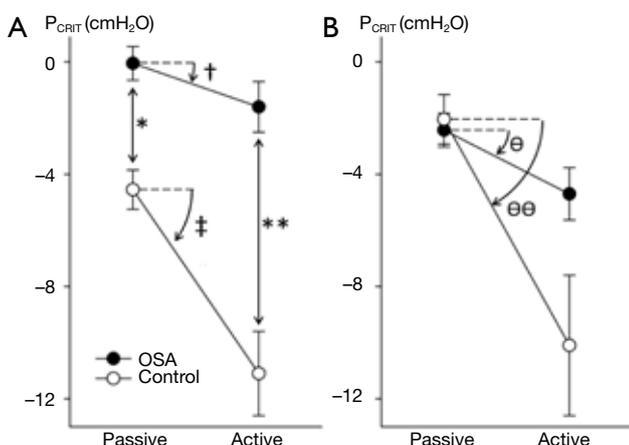
Figure 6 Structural and neuromechanical defects in obstructive sleep apnea (OSA) patients: upper airway critical pressure (P_{CRIT} ± SD) measurements in patients with obstructive sleep apnea (closed circles) and age-, sex-, and body mass index-matched normal controls (open circles) under passive and active conditions. (A) Sleep apnea patients compared to controls demonstrated elevations in both passive P_{CRIT} (*) and active P_{CRIT} (**) compared to controls. Furthermore, under active conditions, OSA patients produced lesser reductions in P_{CRIT} compared to normal controls (see † vs. ‡); (B) among subjects who were matched by structural loads (passive P_{CRIT}), sleep apnea patients lowered the active P_{CRIT} less than normal asymptomatic controls [ΔP_{CRIT} 2.2 cmH$_2$O ±2.2 (Θ) vs. 8.0±7.4 (ΘΘ), respectively]. Adapted from Patil *et al*. 2007 (48).

airflow obstruction could be due to increased pharyngeal compliance. Under these circumstances, reductions in downstream pressure during inspiration can produce increases in linear airflow velocity along streamlines, which further decrease intraluminal pressure (Bernoulli's principle). As intraluminal pressure falls, the airway collapses at its most compliant region [generally located in the velopharynx (35)], forming a choke point with a discontinuity in pressure between the upstream and downstream segments. Elevations in pharyngeal compliance, therefore, can account for the development of inspiratory flow limitation. As the airway wall becomes infinitely compliant, however, its collapsibility is determined by the tissue pressure surrounding the airway rather than the compliance of the airway wall itself. Under these circumstances, the surrounding critical pressure determines airway patency and the severity of airflow obstruction (the Starling resistor, see *Figure 1*). In fact, empiric evidence suggests that the pharynx is a highly compliant structure that approximates the behavior of a Starling resistor, in

which flow limitation develops because surrounding tissue pressures produce a constant back pressure to airflow.

Determinants of upper airway collapsibility

Elevations in P_{CRIT} can be attributed to passive structural defects in the upper airway and disturbances in neuromuscular control (45,46). Utilizing specialized physiologic techniques, investigators have separated structural from neuromuscular components by measuring P_{CRIT} during sleep under conditions of reduced (passive) and elevated (active) neuromuscular activity, respectively (35,47,48). They demonstrated that airway collapsibility was elevated in OSA patients under passive conditions, suggesting underlying anatomic defects in OSA patients compared to age, sex and BMI matched normal controls (see *Figure 6A*). These OSA patients also exhibited blunted active responses to airway obstruction compared to controls, indicating concomitant deficits in pharyngeal neuromuscular control (48). Disturbances in neuromuscular control remained even in those OSA patients whose structural loads were comparable to those of normal controls (see *Figure 6B*). These findings suggest that elevations in P_{CRIT} in OSA patients are due to defects in both upper airway structural and neuromuscular control (*Figure 7*), and those disturbances in both play a pivotal role in OSA pathogenesis. In fact, OSA can only develop when neuromuscular responses do not adequately mitigate the obstruction caused by excess pharyngeal mechanical loads.

Anatomic alterations

Investigators have identified a variety of anatomic factors that contribute to increases in airway collapsibility. Various craniofacial features related to either skeletal morphology or pharyngeal soft tissue may predispose to upper airway collapse. Mandibular size, maxillary height, and hyoid position have been associated with risk for OSA (32,35,49-55). Decreased velopharyngeal area, and tonsillar hypertrophy are soft tissue features that have been associated with increased upper airway collapsibility (35,56). In general, these anatomical variants are thought to increase P_{CRIT} by restricting the size of the boney enclosure around the pharynx and/or increasing the amount of soft tissue contained therein (57).

Obesity and especially abdominal adiposity are also important anatomical risk factors for upper airway obstruction during sleep. The upper airways of obese
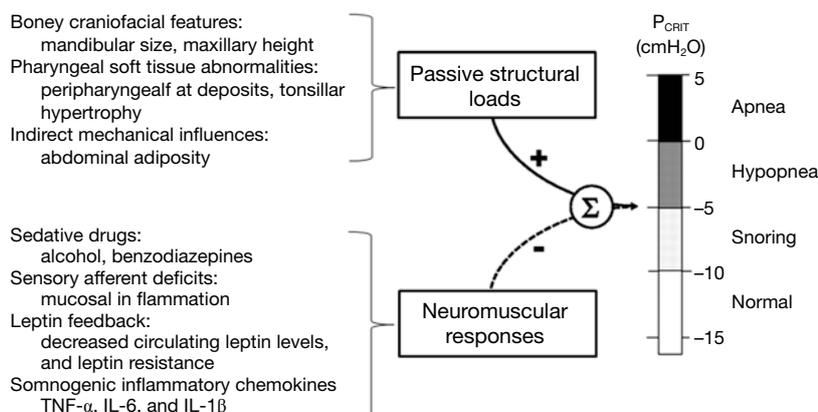
Figure 7 Upper airway collapsibility is the result of the sum of passive structural loads, which increase P_{CRIT}, and neuromuscular responses, which decrease P_{CRIT} and stabilize an inherently collapsible upper airway. Increased airway collapsibility therefore requires defects in both upper airway structure, and neuromuscular responses. Alterations in boney craniofacial features (e.g., retrognathia, decreased maxillary height) and soft tissues (e.g., peripharyngeal fat deposits and tonsillar hypertrophy) may account for structural loads. Defects in neuromuscular responses may arise from sedative drugs, sensory afferent deficits, alterations in leptin levels and/or leptin responses, or somnogenic inflammatory chemokines that may be related to obesity. Adapted from Patil *et al.* 2007 (48).

individuals are more susceptible to collapse (44) and P_{CRIT} increases 1.0 and 1.7 cmH$_2$O per 10 kg/m^2 BMI increase in women and men, respectively (58). Increased fat deposition around the pharynx and airway narrowing (59-61) may increase the extraluminal tissue pressure and augment upper airway collapsibility (62). In addition, lung volumes are decreased in obese persons, leading to decreased caudal traction on the upper airway and an increased critical closing pressure (63-67). These reductions in caudal traction are most pronounced in patients with abdominal adiposity, which can decrease lung volume nearly to the level of residual volume (68-71). Conversely, improvements in OSA with weight loss are likely due to reductions in surrounding tissue pressure and increases in caudal traction, both of which decrease P_{CRIT} (44).

Disturbances in neuromuscular control

Although structural defects play a clear role in the pathogenesis of OSA, these defects may only account for one-third of the variability in OSA severity (72), leaving neuromuscular responses accounting for much of the balance of OSA variability. OSA patients appear to be especially dependent on neuromuscular activity to maintain airway patency and ventilation during sleep (73). This activity varies markedly with reduction in pharyngeal dilator tone at sleep onset that predispose to airway

obstruction (74). Reductions in neuromuscular tone are also suspected to contribute to increased OSA severity during REM compared to NREM sleep in selected patients, and particularly in women and children (75-78). Neuromuscular responses are also influenced by pharmacologic modulators of sleep-wake state (79). Alcohol, sedative medications and hypnotics may decrease active responses to upper airway occlusion, and contribute to upper airway obstruction during sleep. Benzodiazepines have been demonstrated to prolong obstructive apneas and hypopneas (80). The effects of opiate medications on upper airway collapsibility have not been well studied. Nevertheless, blockade of opioid receptors has been demonstrated to decrease P_{CRIT}, which suggests that narcotic medications may increase susceptibility to pharyngeal occlusion (37).

Current evidence also suggests that endogenous neurohumoral agents can modulate upper airway neuromuscular responses. Neurohormonal modulation of pharyngeal neuromuscular activation may in part account for differences in prevalence and severity of OSA between men and women. When matched by BMI, age, and passive mechanical loads, women demonstrated increased neuromuscular compensation and lower disease burden during NREM sleep compared to men (81). Sex differences in neuromuscular control may well be due elevations in circulating leptin levels in women compared to men (82,83). With weight loss, P_{CRIT} falls by 6.2 cmH$_2$O per 10 kg/m^2
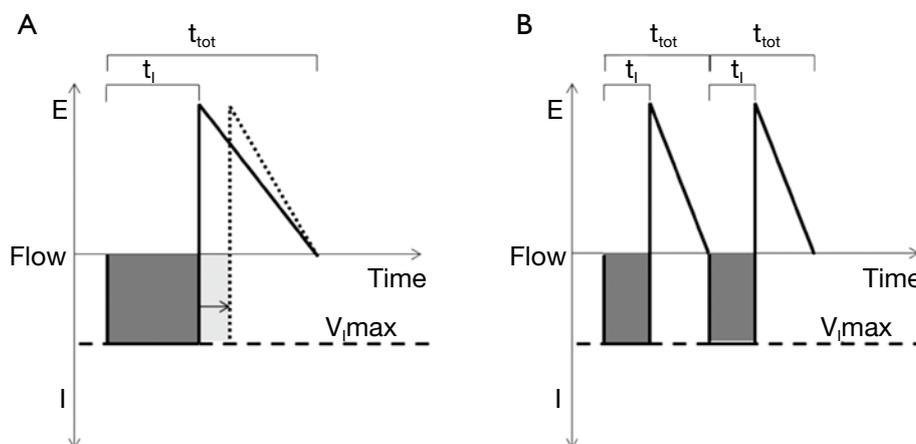
Figure 8 Flow profiles during inspiratory flow limitation. Positive flow represents expiration [E], whereas negative flow represents inspiration [I]. (A) During inspiratory flow limitation, minute ventilation (dark shaded area) can be approximated by the product of maximal inspiratory flow ($V_I$max, dashed line) and inspiratory time (t_I). Minute ventilation can be increased by increasing inspiratory duty cycle (IDC) (t_I/t_{tot}), as demonstrated by the arrow and dotted line; (B) in contrast, increasing the respiratory rate does not increase minute ventilation (the sum of dark shaded areas in B is equal to A) when t_I/t_{tot} remains constant. Adapted from Schneider *et al.* 2009 (108).

decrease in BMI in apneic men (44), which is greater than the above noted 1.7 cmH$_2$O increase in passive P_{CRIT} per 10 kg/m^2 increase in BMI attributable to weight gain (58). These observations suggest that obesity may increase upper airway collapsibility through alterations in pharyngeal neuromuscular responses in addition to imposing increased anatomical loads. Investigations have demonstrated elevations in circulating levels of somnogenic inflammatory chemokines, specifically TNF-α, TNF-α receptor I, IL-6, and IL-1β in association with obesity, which may account for the decreases in neuromuscular activity in obesity and OSA patients (84-97).

Pharyngeal neuromuscular activity is also controlled by chemical and mechanical reflexes. Hypercapnia is also a potent stimulator of upper airway neuromuscular activity, which decreases P_{CRIT} (98-100). Hypocapnia, on the other hand, produces a relatively passive state, and is associated with elevations in P_{CRIT}. The upper airway demonstrates decreased collapsibility during expiration compared to inspiration due to phasic activation of pharyngeal muscles (101). Phasic volume feedback, which is mediated by pulmonary stretch receptors, can also inhibit upper airway neuromuscular activity and increase P_{CRIT} (102,103). Pharyngeal sensory afferents can detect intraluminal negative pressure swings during airflow obstruction, and recruit neuromuscular activity (104). Pharyngeal sensory inhibition with topical analgesics has been demonstrated

to decrease these neuromuscular responses to upper airway obstruction (105,106). Similarly, mucosal inflammation may blunt local afferents and neuromuscular responses to upper airway obstruction, leading to worsening upper airway obstruction during sleep (107).

Responses in respiratory timing can further augment reflex responses to upper airway obstruction and stabilize ventilation during sleep. The inspiratory duty cycle (IDC), which is the ratio of inspiratory time to total respiratory cycle time, is the most significant determinant of ventilation during periods of inspiratory flow limitation (81,108,109) (see *Figure 8*). In response to pharyngeal obstruction during sleep, IDC increases nearly immediately with resultant increases in ventilation (108,110,111). In contrast, respiratory rate does not significantly alter minute ventilation in the flow-limited state. Thus, increases in pharyngeal neuromuscular activation and IDC can help maintain ventilation during periods of inspiratory flow limitation and will stabilize respiratory patterns accordingly (see below).

Modeling the oscillatory patterns in OSA

Thus far, we have examined factors that promote airway collapse at the onset of obstructive sleep disordered breathing events. To explain the repetitive nature of OSA events, investigators have cast the respiratory system as a closed-loop control system and have elucidated fundamental
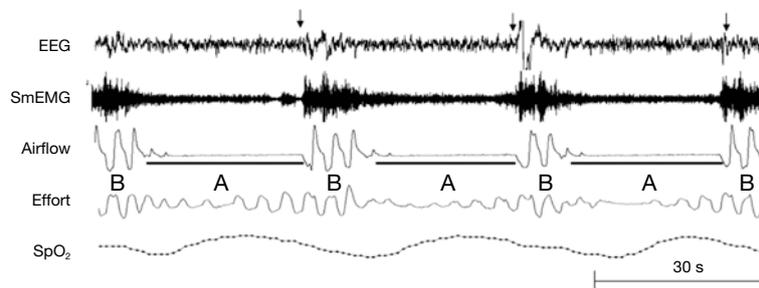
Figure 9 A nocturnal polysomnogram of a patient with sleep apnea demonstrates recurrent apneas (black bars) and arousals (arrows). Rather than smooth oscillations in respiratory patterns, airflow is characterized by abrupt transitions between periods of (A) upper airway obstruction (apneas) during sleep and (B) upper airway patency during arousals.

determinants of these oscillatory patterns. Crowell and colleagues first described the influence of circulatory delay on the periodic breathing. By inducing feedback delay between the central circulation and chemoreceptors in the dog, they reproduced the periodic respiratory pattern of Cheyne-Stokes respirations (112). Cherniack and colleagues highlighted the effects of hypocapnia which lead to apnea and subsequent periodic breathing patterns (113). They further expanded on our understanding of ventilatory control and periodic breathing with the development mathematical models that featured chemosensitivity as a predictor of unstable respiratory patterns (114,115). Khoo and colleagues extended this methodology by incorporating transitions in sleep-wake state and arousal phenomena into mathematical models of periodic breathing (116). In these models, chemosensitivity and ventilatory efficiency were summarized by a singular term, loop gain, to describe the propensity towards oscillations in respiratory patterns. Younes and colleagues manipulated loop gain experimentally with proportional assist ventilators, and demonstrated that elevations in loop gain can cause periodic breathing (117). In cross-sectional studies by Wellman and colleagues, loop gain was identified as one of several possible determinants of sleep apnea pathogenesis (118,119). Studies by these investigators have also suggested that pharmacological manipulation of loop gain and arousal threshold can ameliorate OSA (120-122).

Two key assumptions underlie traditional approaches to mathematical models of periodic breathing patterns. The first is that ventilatory responses change in proportion to alterations in ventilatory demand. For instance, hypoventilation, which results in elevations in CO_2, would lead to proportionate increases in ventilatory drive. The second is that the mechanical components of the respiratory

system and the control of ventilation remain relatively constant across cycles of periodic breathing. These two principles—linearity and time-invariance (123)—define linear control systems, which can be described by mathematical models that accurately predict smooth sinusoidal oscillations as those observed in Cheyne-Stokes respirations.

Rather than smooth oscillations in airflow, OSA appears to be characterized by abrupt transitions in upper airway patency and ventilation, which suggest a striking departure from the principles of linearity and time-invariance (see *Figure 9*). Remmers *et al.* demonstrated that obstructive apneas are characterized by the development of dynamic pharyngeal obstruction during sleep with prompt re-opening upon arousal, suggesting marked state-dependence in pharyngeal neuromuscular activity and upper airway collapsibility (17). In fact, upper airway patency is suddenly restored at apnea termination as it shifts from a flow-limited state to a non-flow-limited state. The abrupt termination of these sleep disordered breathing events can be modelled as a switch in an electrical analog of sleep disordered breathing (see *Figure 10*). When this shift occurs, ventilation can once again track ventilatory drive. Ventilatory drive, in turn, responds to changes in mechanical and chemical afferent inputs, which detect differences between ventilatory supply and demand (left and right sides of *Figure 10*). At sleep onset, however, the upper airway transitions to a flow limited state. Under these circumstances, ventilation is determined by the degree of upper airway patency rather than ventilatory drive. If ventilatory supply no longer matches demand, ventilatory drive progressively increases (see marker ① in *Figure 11*). These increases can be associated with alterations in upper airway patency and ventilatory timing that help mitigate the obstruction and/or restore ventilation
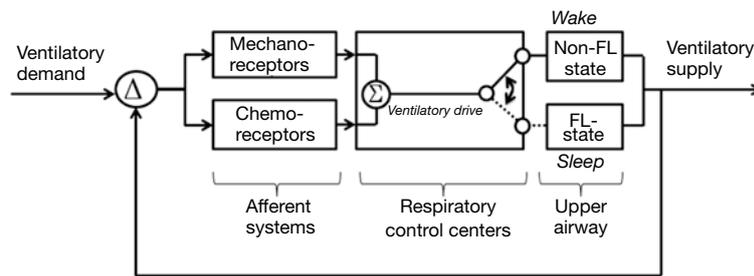
Figure 10 Upper airway transition model for obstructive sleep apnea (OSA). In OSA, the upper airway alternates between states of flow limitation during sleep, and non-flow-limited breathing during wakefulness. During sleep, a flow limited upper airway caps ventilatory supply, which dissociates from ventilatory drive (see dashed line). When ventilatory supply does not meet demand, differences (Δ) are detected by mechanical and chemical receptors and respiratory control centers, leading to increases in ventilatory drive. Increases in ventilatory drive during periods of flow limitation trigger arousal from sleep with restoration of upper airway patency.
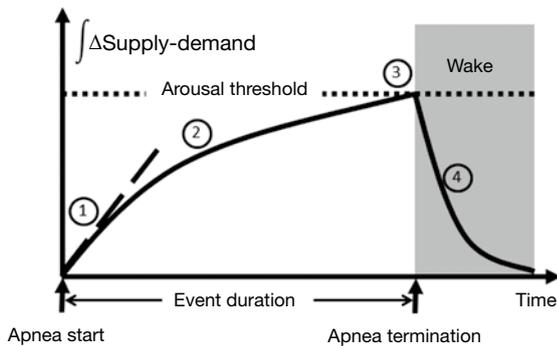
Figure 11 Ventilatory supply-demand dynamics. 1. At the start of an obstructive event, cumulative supply-demand difference (see Δ in *Figure 10*) is initially determined by the degree of upper airway obstruction (supply) and CO_2 production (demand); 2. rate of accumulation may be decreased by neuromuscular responses and increase in ventilatory efficiency as CO_2 rises; 3. when supply-demand difference meets a threshold, arousal occurs and terminates the apnea; 4. pent-up ventilatory demand is relieved due to improved upper airway patency during the arousal.

(marker ② in *Figure 11*). If neuromuscular and ventilatory timing mechanisms do not adequately restore ventilation, ventilatory supply-demand mismatch continues, leading to progressive increases in ventilatory drive. Once drive exceeds a threshold, arousal is triggered (marker ③ in *Figure 11*), relieving pent-up ventilatory demand as the airway transitions to a non-flow-limited state (maker ④ in *Figure 11*). In fact, repetitive transitions have been demonstrated when ventilation decreases by more than 12-20% of baseline or $V_I$max falls below 250 mL/s (81,124). The aforementioned

model suggests that state-dependent alterations in upper airway patency and resulting mismatch between ventilatory supply and demand play pivotal roles in OSA pathogenesis.

In this model, several factors can influence the overall evolution and severity of obstructive breathing episodes. In *Figure 11*, we plot the cumulative difference between ventilatory supply and demand over time during an obstructive sleep disordered breathing event. In general, the duration of sleep disordered breathing events is governed by the time required for cumulative supply-demand mismatch to reach the arousal threshold. At the onset of sleep disordered breathing episodes, the initial shortfall in ventilation is determined by the severity of upper airway obstruction. As ventilation falls, CO_2 rises in proportion to the decrease in alveolar ventilation and the metabolic rate (CO_2 production). Metabolic rate, in turn, is determined by body mass and composition, food sources (e.g., respiratory quotient), basal metabolic rate, sex and work of breathing at rest. Inspiratory flow limitation can further increase the work of breathing and thereby widen ventilatory supply-demand mismatch. Under these circumstances, minute ventilation must increase well above that in the non-flow limit state to satisfy the additional ventilatory demand and stabilize breathing patterns. Supply-demand dynamics can also be affected by ventilatory efficiency. Decreases in ventilatory efficiency due to increased dead space and cardiopulmonary disease will accelerate the development of supply-demand differences. Along with the severity of upper airway obstruction, excess metabolic demand and underlying cardiopulmonary disease can increase the overall severity of sleep disordered breathing. In contrast, sleep disordered breathing events may be prolonged by factors

that slow the development of supply-demand differences, including compensatory neuromuscular mechanisms that mitigate upper airway obstruction and augment ventilation. Sleep disordered breathing events can also be prolonged by raising the arousal threshold with sleep deprivation (125) or hypnotic agents (121,122). Because upper airway neuromuscular control is highly dependent upon sleep wake state, increasing arousal threshold will tend to increase the degree of hypoventilation and oxyhemoglobin desaturation during sleep, as well (79). On the other hand, lowering the arousal threshold may shorten events and increase sleep disruption while minimizing alterations in gas exchange. Thus, the arousal threshold and ventilatory supply-demand dynamics can interact to modulate the overall polysomnographic expression of OSA.

Conclusions

Upper airway obstruction is essential in the pathogenesis of OSA. OSA is largely absent in those individuals without an inherently collapsible upper airway on a structural basis. When P_{CRIT} exceeds -5 cmH$_2$O, the risk for OSA markedly increases. The appearance of OSA features parallels the rise in P_{CRIT}, increasing from simple snoring, to cyclic hypopneas, and then to fully occlusive apneas. These features are recapitulated in normal persons when upper airway obstruction is induced, and are abolished in OSA patients when airway patency is restored. Therefore, upper airway obstruction alone constitutes both a necessary and sufficient condition for the development of OSA.

Once the airway has collapsed, several factors modify the response to airway obstruction, and affect the ultimate expression of sleep disordered breathing. Neuromuscular responses preserve ventilation and protect against the development of OSA. When neuromuscular compensatory mechanisms are insufficient for a given structural load, ventilatory demand and ventilation dissociate and repeated sleep disordered breathing events ensue. Trade-offs between sleep stability and ventilation can result in a full range of OSA severity and expression. Recurrent arousals and transient increases in airway patency may restore ventilation between periods of sleep, while alterations in neuromuscular responses to upper airway obstruction may improve sleep stability at still suboptimal levels of ventilation.

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Footnote

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Diagnostic bronchoscopy—current and future perspectives

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Abstract: Lung cancer is the leading cause of cancer-related mortality worldwide. Standard bronchoscopy has limited ability to accurately localise and biopsy pulmonary lesions that cannot be directly visualised. The field of advanced diagnostic bronchoscopy is rapidly evolving due to advances in electronics and miniaturisation. Bronchoscopes with smaller outer working diameters, coupled with miniature radial and convex ultrasound probes, allow accurate central and peripheral pulmonary lesion localisation and biopsy while at the same time avoiding vascular structures. Increases in computational processing power allow three-dimensional reconstruction of computed tomographic raw data to enable virtual bronchoscopy (VB), providing the bronchoscopist with a preview of the bronchoscopy prior to the procedure. Navigational bronchoscopy enables targeting of peripheral pulmonary lesions (PPLs) via a "roadmap", similar to in-car global positioning systems. Analysis of lesions on a cellular level is now possible with techniques such as optical coherence tomography (OCT) and confocal microscopy (CM). All these tools will hopefully allow earlier and safer lung cancer diagnosis and in turn better patient outcomes. This article describes these new bronchoscopic techniques and reviews the relevant literature.

Keywords: Lung cancer; bronchoscopy; diagnosis

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Overall 5-year survival is poor at 15% with little improvement over the last twenty years (1). Lung cancer presenting at a late stage is largely responsible for this poor survival; only 20% are potentially curative with surgical resection. Anti-smoking campaigns and government legislation will reduce lung cancer burden in younger generations but a significant proportion of the older generation who have smoked in the past remain at risk.

Innovative bronchoscopic techniques diagnose lung cancer earlier and more accurately to improve patient outcomes. Narrow band imaging (NBI) and autofluorescence bronchoscopy (AFB) detect pre-invasive malignancy. Endobronchial ultrasound (EBUS) and Electromagnetic Navigation Bronchoscopy (ENB)

are safer alternatives to mediastinoscopy and computed tomography guided transthoracic needle aspiration (CT-TTNA). Emerging technologies such as optical coherence tomography (OCT) and confocal microscopy (CM) allow lesional assessment on a cellular level.

This article describes these technologies and explains how they enhance lung cancer diagnosis and staging.

Standard bronchoscopy

Gustav Killian invented the rigid bronchoscope in 1898. In 1967 Ikeda pioneered the flexible bronchoscope, and video bronchoscopes became mainstream in the early 1980's. Despite quickly becoming the cornerstone of lung cancer diagnosis, the shortcomings of white light bronchoscopy (WLB) are immediately obvious. Due to the branching nature of the bronchial tree, the airway diameter

rapidly reduces the more distal one advances towards lung periphery. A standard 5.9 mm bronchoscope can only access the 4-5th generation bronchi with visualization of the next 1-2 generations, covering only 1/3 of the approximately 23-generation bronchial tree. The majority of lesions visualized on CT are therefore beyond direct bronchoscopic vision. Aberrations in bronchial mucosa, such as angiogenic squamous dysplasia (ASD) or squamous cell carcinoma (SqCC) *in situ* (CIS) are indistinct and easily missed when lit by white light (2). Similarly extra-bronchial structures such as mediastinal lymph nodes cannot be visualized, and blind needle aspiration (cTBNA) is possible for a limited number of nodal stations (3).

Meta-analysis by Rivera from studies between 1970-2001 found the sensitivity and specificity for *bronchoscopically visible* lesions was 88% and 100% respectively (4). Diagnostic accuracy for *bronchoscopically invisible* lesions under fluroscopic guidance varied from 36% to 88%, dependent on biopsy method (transbronchial biopsy *vs.* cytology brush *vs.* bronchoalveolar lavage), the number of samples taken, and lesion size. Yield was most affected by lesion size-sensitivity for peripheral pulmonary lesions (PPLs) >2 cm was 63%, decreasing to 34% for PPLs <2 cm. Whilst CT-TTNA is undoubtably more accurate than WLB (pooled sensitivity 0.9; 95% confidence interval 0.88-0.91) its complication rate is higher (5).

Thus, WLB is effective at diagnosing bronchoscopically *visible* lesions, but is limited in diagnosing bronchoscopically *invisible* lesions, *in situ* tumours, and for mediastinal staging of lung cancer.

Endobronchial/mucosal lesions

AFB

AFB takes advantage of endogenous fluorophores in bronchial tissue to inform about metabolic state and biochemical composition of tissues. Normal bronchial tissue fluoresces strongly in green when illuminated by violet or blue light, however as the epithelium becomes dysplastic, progresses to *in situ* carcinoma and finally to invasive cancer, the amount of green autofluorescence decreases and red fluorescence also decreases although to a lesser degree. These abnormal areas contrast sharply with normal mucosa.

Two meta-analyses have studied the value of AFB combined with WLB versus WLB alone for detection of intraepithelial neoplasia and invasive lung cancer. The first

(14 studies, n=1,358) demonstrated a pooled sensitivity and specificity of AFB + WLB of 0.9 and 0.56, compared to 0.66 and 0.69 for WLB alone (6). The second (21 studies, n=3,266) showed the relative sensitivity on a per lesion basis of AFB + WLB versus WLB alone to detect CIS and invasive cancer was 2.04 and 1.15 respectively (2). AFB + WLB is less specific than WLB alone because false positives are common with AFB due to inflammation, mucous gland hyperplasia and inter-observer error, however specificity and inter- and intra-observer variation can be improved to 80% by combining the quantitative red/green fluorescence ratio (R/G) with bronchoscopic findings (7).

NBI

NBI visualizes bronchial mucosa with blue light (415 nanometers) and green light (540 nanometers) to accentuate superficial capillaries and deeper submucosal vessels respectively, while at the same time reducing light scatter from other wavelengths seen with white light. NBI detects the characteristic abnormal angiogenesis associated with dysplastic lesions. Most of the data detailing the benefits of NBI are from gastroenterological studies and head and neck cancers, however pulmonologists are increasingly using NBI to detect early stage bronchial mucosal lesions.

In 2003 Shibuya *et al.* studied 48 patients with sputum cytology suspicious or positive for malignancy (8). These patients underwent WLB and AFB, with suspicious areas subsequently interrogated with NBI of differing wavelengths and biopsied for histology. Dotted vessel diameter seen on NBI-B1 (400-430 nm) most closely agreed with pathological examination of ASD vessel diameter.

Shibuya *et al.* also studied differing NBI characteristics of ASD, CIS, micro-invasive tumour, and invasive SqCC (9). By identifying tortuous vessel networks, dotted vessels, and spiral and screw type vessels, the authors could confidently differentiate between the different stages of multistep carcinogenesis of SqCC.

NBI has a higher specificity and equivalent sensitivity to AFB. Herth *et al.* evaluated diagnostic yields of NBI alone, and combined with AFB and WLB, in 62 patients referred for airway cancer screening (10). All abnormal lesions underwent forceps biopsy. NBI was less sensitive but more specific than AFB, when compared to WLB. There was no benefit in combining AFI and NBI, a finding confirmed in a more recent study (11). NBI detects dysplasia or malignancy in 23% of patients with normal WLB (12). NBI after WLB

led to a change in therapeutic decisions in approximately 10% of patients (13). It is uncertain how NBI compares to high definition WLB (which provides substantially higher resolution images than conventional WLB) for detection of early bronchial mucosal abnormalities.

The natural history of pre-invasive malignancy remains uncertain and treatment at this early stage has not been shown to improve survival however it is likely that at least some of these lesions, if left untreated, will progress to invasive carcinoma. AFB and NBI have higher sensitivity and specificity for detecting mucosal lesions compared to WLB, and diagnostic bronchoscopists should be familiar with their use.

OCT

OCT provides cellular imaging at and below the tissue surface (14-16). It was developed in the 1990's for ophthalmic applications but has since been used to assess vessel structure, atherosclerotic plaque, and more recently, bronchial wall structure.

Light is emitted by an imaging catheter and the interference pattern between reflected light and light backscattered from the tissue at different depths is collected and analyzed by an interferometer. These patterns are then recombined and decoded, forming a high resolution cross-sectional image. Contact between instrument and tissue is unnecessary and intravenous contrast, dyes or radiation are not needed. OCT resolution is 20 times higher than ultrasound and can be displayed on a monitor in real time. OCT imaging depth is 2-3 mm, and axial and lateral resolution varies between 5-30 micrometers depending on the scanning conditions.

Tsuboi *et al.* compared OCT images to histological findings of bronchial lesions (14) and found that on OCT, normal bronchial mucosa appears homogeneous whereas the submucosal layer is reflective due to extracellular matrix; A gap is visible between the submucosa and smooth muscle layer, and underlying cartilage shows much scattering. Alveoli have a uniform bronchial wall appearance and air-containing alveoli can be clearly differentiated. Infiltrating cancers, on the other hand, show unevenly distributed high backscattering areas and loss of layer structure and glandular tissue. Lam *et al.* demonstrated that quantitative measurement of epithelial thickness could differentiate between invasive carcinoma and CIS (P=0.004), and also between dysplasia and metaplasia or hyperplasia (P=0.002). Basement membrane remained intact with CIS, but

became disrupted with invasive cancer (15). Certain OCT characteristics can potentially differentiate SqCC from adenocarcinoma without the need for biopsy (17).

Despite these promising pilot studies, it remains to be seen if and how OCT will add value to our current diagnostic approaches. Possible uses for OCT include: distinguishing benign from malignant central and peripheral lesions, differentiating CIS from mincr-invasive cancer; and improving bronchoscopic sampling of PPLs. The performance characteristics and limitations of OCT need to be defined before the technique becomes mainstream.

PPLs

EBUS radial probe (RP-EBUS)

Advances in electronic miniaturization allow a 360-degree viewing ultrasound to fit into a 1.4 mm probe that can be passed through the working channel of a standard bronchoscope. This so-called RP-EBUS allows localization of peripheral lesions and depth assessment of endobronchial lesions. At standard frequency of 20 MHz, the spatial resolution is less than 1 mm and penetration depth is 4-5 cm.

RP-EBUS has two main uses.

Localisation of PPLs

By placing the ultrasound probe into lung periphery one can characterize the tissue densities surrounding the probe. Normal air-filled alveoli have a homogeneous "snow-storm" appearance. If the probe is within a solid lesion however the interface between the mass and surrounding aerated lung is represented by a bright line, confirming the probe is within the target.

The use of a guide sheath (GS) in combination with RP-EBUS was introduced in 2004 (18). The GS is a catheter that fits over the RP-EBUS, leaving only the distal ultrasound probe exposed. The RP-EBUS/GS are advanced together through the working channel into the target subsegment until the lesion of interest is localized. Advancing and retracting the RP-EBUS/GS defines the lesion's proximal and distal extents. Once the desired biopsy site is established, the GS is left *in situ* and the RP-EBUS is removed and replaced by pre-measured biopsy tools, ensuring samples are taken from the desired location. An additional theoretical benefit of GS is tamponade of biopsy-related bleeding (*Figure 1*).

Perhaps the best evidence favouring RP-EBUS/ GS over fluoroscopy guided transbronchial lung biopsy
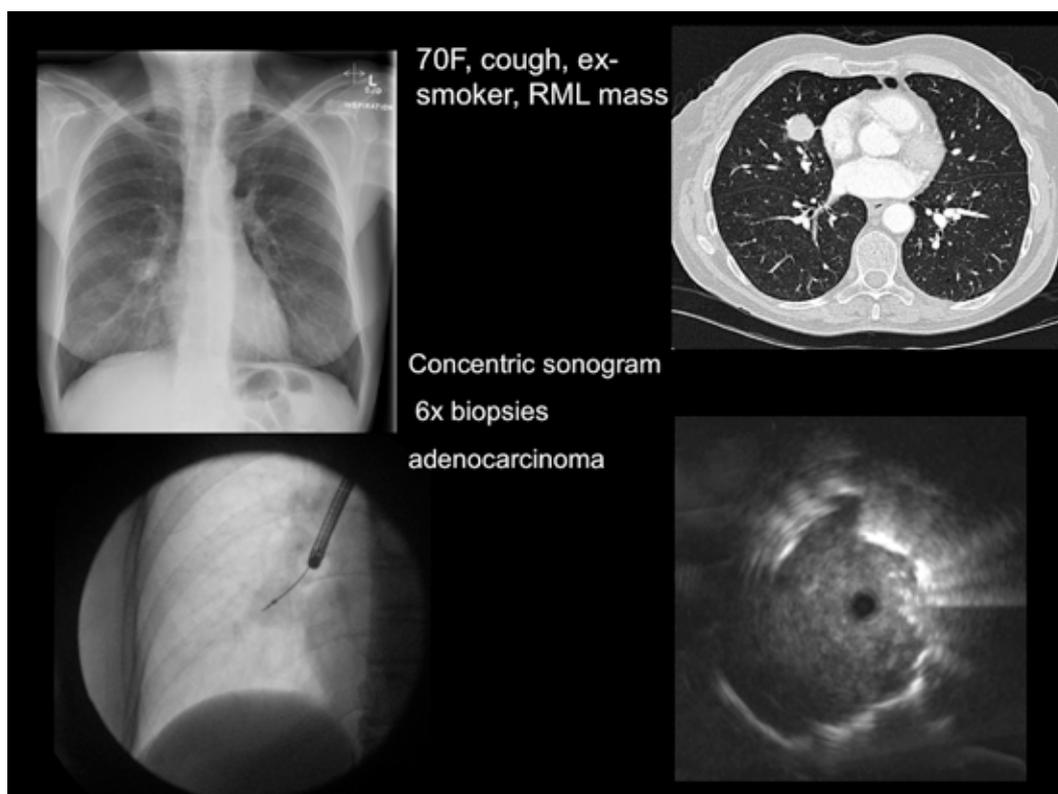
Figure 1 Example of RP-EBUS being used to diagnose a peripheral pulmonary lesion. Note the well demarcated outline of the lesion and the concentric nature of the image in the bottom right panel.

(TBLBx) for PPLs was published in 2005 by Paone (19). 221 patients with PPLs were randomly assigned to either procedure (97 RP-EBUS/GS, 124 TBLBx) and those without a diagnosis underwent more invasive investigation. Sensitivity for lung cancer was 0.79 and 0.55 (P=0.004), and accuracy was 0.85 and 0.69 (P=0.007) in RP-EBUS/GS and TBLBx groups respectively, with the majority of the benefit evident in lesions <3 cm [sensitivity of RP-EBUS/GS vs. TBLBx for <3 cm, 71% (47-95%) vs. 23% (3-43%), P value <0.001].

A systematic review and meta-analysis of RP-EBUS/GS for PPL diagnosis (16 studies n=1,420 patients) showed point sensitivity for diagnosis of lung cancer was 0.73, however there was significant study heterogeneity. Complication rates varied between 0-7.4%, with the most common being minor bleeding. Pooled pneumothorax rate was 1.0%, and rate of intercostal drainage was only 0.4% (20).

Most RP-EBUS/GS data originate from centres with bronchoscopic expertise and hence may not reflect "real-world" results. Roth *et al.* performed a prospective randomised cohort study of RP-EBUS/GS for PPLs by 29 different physicians practicing at community hospitals in Western Norway between June 2005 and January 2008 (21). With a cancer prevalence of 71.5%, the sensitivity for malignancy in the RP-EBUS/GS group was 36% compared with 43.7% in the non-EBUS group. If there was a bronchus leading directly to the lesion, the diagnostic sensitivity was considerably higher (62.2%). This study may provide a more realistic view of what is achievable with RP-EBUS/GS in a community hospital and demonstrates that EBUS is useful in confirming lesion location, but not in guiding the bronchoscopist to the lesion.

The only consistent predictor of success is probe location in relation to the lesion; If the probe is surrounded by tumour (concentric ultrasound image) the diagnostic yield is much higher than if the probe is adjacent to (eccentric image) or not associated with the target lesion (18,22-24). Although Kurimoto found yield was independent of lesion size (18), subsequent studies recognise that higher diagnostic yields are achieved from larger lesions (20).

Few studies have compared RP-EBUS/GS to the non-invasive gold standard of CT-TTNA. In 2008 Fielding

described a prospective series of RP-EBUS/GS and compared this to a retrospective review of CT-TTNA during the same period. Diagnostic sensitivity for RP-EBUS/GS was only 35% for lesions touching the visceral pleura, compared to 74% for lesions not touching the visceral pleural. While overall pneumothorax rates were 1% and 28% in EBUS GS and CT groups respectively, the CT-TTNA pneumothorax rate was only 2.6% for lesions in contact with the visceral pleura (25). These results suggest that CT-TTNA rather than RP-EBUS/GS should be the first line investigation for pleural-based lesions.

Depth of invasion of endobronchial lesions

RP-EBUS with a surrounding inflatable balloon can clearly define tracheal and bronchial wall layers, making it an excellent tool to assess tumour invasion. Ultrasonographic assessment of tumour depth correlates well with histopathologic findings (26) and this measurement determines appropriate therapy; Tumours that invade through the cartilage layer require radiotherapy or surgery, whereas those with an intact cartilage layer can be treated endoscopically.

RP-EBUS can also determine whether centrally located tumours adjacent to the trachea are invading the trachea (clinical T4 stage) or simply adjacent to and compressing but not invading the trachea (clinical T1a-3 depending on lesion size). Herth *et al.* studied 131 consecutive patients with central thoracic malignancies potentially involving the central airways (27). All patients underwent chest CT followed by WLB and RP-EBUS, with subsequent surgical evaluation and radiology results blinded from the bronchoscopists and surgeons. CT reported 77% of lesions were invading airways, but RP-EBUS showed invasion in only 47% of cases. When using surgical assessment as the gold standard, RP-EBUS had a specificity of 100%, sensitivity of 89%, and accuracy of 94%, for assessing tumour invasion.

ENB

ENB is a relatively new bronchoscopic technique with both diagnostic and therapeutic applications. ENB is a two-stage process: pre-procedure planning, and the actual procedure itself. DICOM data is uploaded to a planning computer via network or compact disk. The planning screen consists of four windows, each of which can display axial, sagittal or coronal views, as well as a virtual bronchoscopic animation and three-dimensional bronchial tree. The bronchoscopist outlines the target and then places waypoints along bronchi that lead to this lesion. The planned path can then be viewed via virtual bronchoscopic animation, allowing the operator to see precisely which sequence of airways lead to the lesion.

Selective cannulation of bronchi is possible with a specialized cannula housed in an extended working channel (EWC), passed together through the working channel. The proximal end houses a "steering wheel" that allows deflection of the distal tip in one of eight directions; the distal end contains a "locatable guide" (LG), whose position is tracked through an electromagnetic field encompassing the patient's chest with the assistance of three location pads placed on the patient's chest. The computer provides instructions on how and when to turn the "steering wheel" and advance the catheter to reach each waypoint and finally the target lesion (*Figure 2*). Once the LG is in close proximity and aligned to the target lesion, the EWC is left in place and the LG is removed and replaced with biopsy instruments.

Most of the published ENB literature is case series of patients with PPLs. The overall diagnostic yield for ENB alone is highly variable and ranges from 59% to 77.3% (28-34). The only randomised controlled trial compared (31) EBUS RP, ENB, or a combined approach (ENB to navigate to the lesion and RP-EBUS to confirm lesion localization) to diagnose one hundred and twenty PPLs. Diagnostic yield was 69%, 59% and 88% for RP-EBUS, ENB, and combined ENB/RP-EBUS groups respectively, suggesting that highest diagnostic yield may be achieved via combined procedures that utilize the strengths of each modality.

The majority of ENB publications are non-consecutive cohorts that do not describe selection/inclusion criteria, and (apart from Eberhardt *et al.*) do not randomize patients to competing modalities. ENB has never been compared to CT-TTNA and thus it is uncertain where ENB fits into the diagnostic algorithm. Due to its high cost and considerable pre-procedure planning (both to obtain DICOM images of recommended parameters, as well as pathway planning) ENB is only likely to become mainstream if consumable prices fall and high level evidence demonstrates diagnostic equivalence to CT-TTNA and/or additional benefit above RP-EBUS. Based on Eberhardt's study, ENB and RP-EBUS may have complimentary roles, however this combined approach would likely increase cost and procedure time.
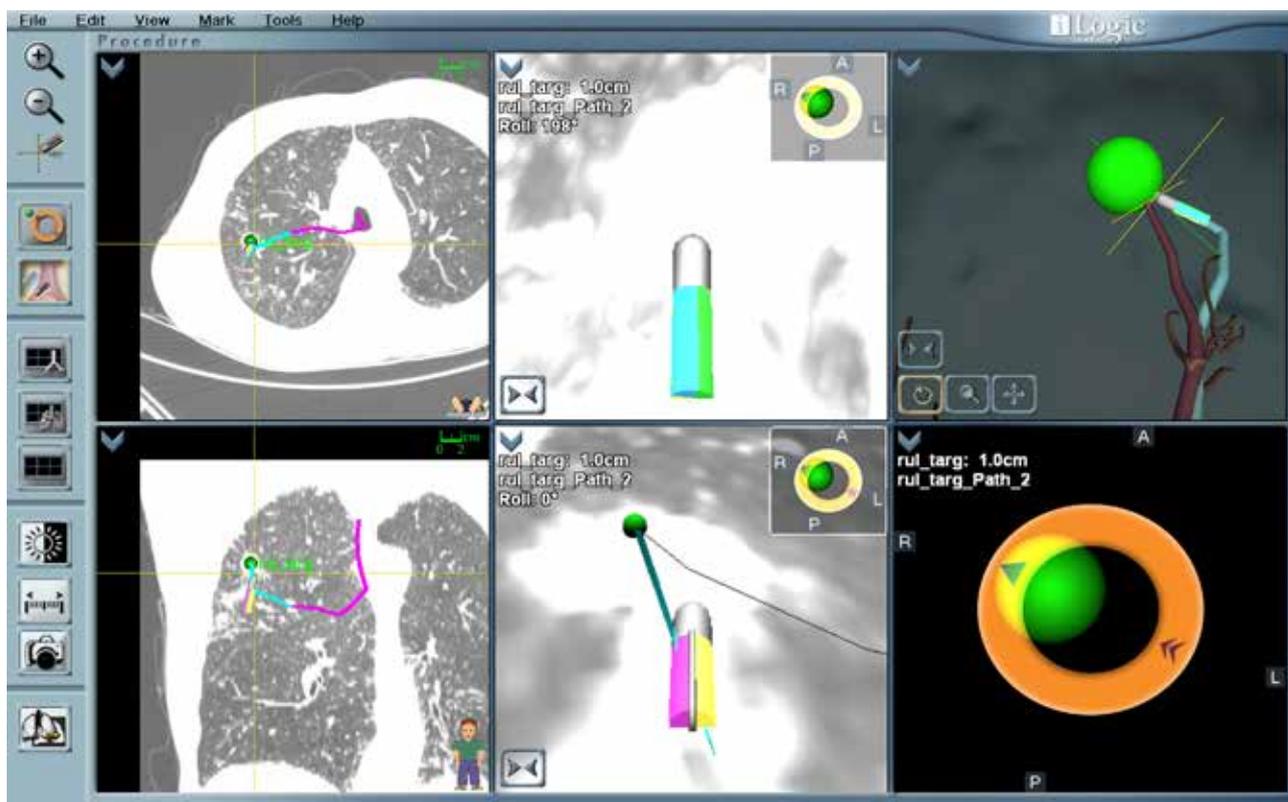
Figure 2 ENB procedure screen showing 6 viewports. Top right = dynamic 3D map; bottom right = tip view (green sphere = target); bottom centre-local view; top centre = maximal image projection view.

Virtual bronchoscopy (VB)/ultrathin bronchoscopy (UB)

VB aims to address the inability of RP-EBUS to guide the bronchoscopist to the target lesion. Traditionally, the bronchoscopist views two-dimensional axial, coronal, and sagittal CT views, and mentally reconstructs a three-dimensional image of the bronchial tree before plotting a path to the target lesion. VB allows CT reconstruction of the bronchial tree allowing "virtual" bronchoscopic animation enabling more accurate procedure planning. An example of this was shown in *Figure 3*.

For maximum utility, VB should be coupled with pathway planning software (for example, "Lungpoint", Broncus Medical Inc, CA, USA) and UB; Newer scopes have external diameters of only 2.8 mm (35), and allow direct visualization up to the 9th generation bronchus. A direct consequence of UB however is a smaller working channel and hence smaller biopsy samples.

UB was initially used by Asano to perform barium marking before thoracoscopic surgery for PPLs (36). The UB could be guided under direct vision to a median sixth generation bronchi (range 4th-9th generation) and markers could be placed to a median distance of 4 mm from the lesion (within 10 mm in 27 of 31 lesions). A separate study whereby patients underwent both WLB and UB in the same procedure demonstrated diagnostic rates of 54.3% and 60% respectively, and when both were combined the yield increased further to 62.8% (37). UB was able to obtain diagnostic material in 59.3% of the patients who had negative rapid cytology on WLB.

Asahina *et al.* assessed the utility of combining VB with RP-EBUS/GS in 29 patients with PPLs ≤30 mm. 80% of lesions were visualized ultrasonographically and diagnostic sensitivities were 44.4% for lesions <20 mm, and 91.7% for lesions ≥20 mm (38). In a randomised trial of 199 patients with PPLs ≤30 mm undergoing RP-EBUS/GS with and without VB (VBNA *vs.* non-VBNA groups) the VBNA group demonstrated higher diagnostic yield (80.4% *vs.* 67%, P=0.032), shorter procedure time (24.0 *vs.* 26.2 mins, P=0.016), and shorter navigation time (8.1 *vs.* 9.8 mins, P=0.045) (39).

Figure 3 An example of virtual bronchoscopy. (A) Lesion in right upper lobe; (B) Real time virtual image overlay to guide the pathway to the tumour; (C) Virtual bronchoscopic image.

Recently though a randomised controlled multicentre trial of UB with and without VB for PPLs found no difference in diagnostic yield. 350 patients were randomised and yields were 67.1% *vs.* 59.9% for VNBA *vs.* non-VBNA groups respectively. Subgroup analysis showed improved yield in the VBNA group for right upper lobe lesions, lesions invisible on CXR, and lesions in the peripheral third of the lung field. It could be argued that VB is of little benefit to highly experienced operators, however it still may be of significant assistance in those less familiar with bronchial anatomy (40).

UB/VB has three major shortcomings. Firstly, the ability to obtain sufficient tissue for molecular analysis with UB is unknown but presumably reduced as biopsy forceps size may affect biopsy size and quality. Secondly, VB quality is dependent on CT source data and recommended DICOM parameters may be unavailable. Thirdly, VB systems rely on a skilled second operator to manipulate the VB image to the same orientation as the real-time bronchoscopic image; without this, the risk of disorientation is high (41).

Complications of guided bronchoscopic techniques

The biggest advantage of bronchoscopic methods over CT-TTNA for biopsy of PPLs is the lower complication rate. In a meta-analysis of RP-EBUS (16 studies, n=1,420), complication rates varied between 0-7.4%. The pooled pneumothorax rate was 1.0% and the pooled rate of intercostal catheter drainage was 0.4%. No patients had bleeding requiring intervention, and no deaths were reported.

In contrast, data from 15,865 adults who underwent CT-TTNA from the 2006 Healthcare Cost and Utilisation Project's State Ambulatory Surgery Databases and State Inpatient Databases for California, Florida, Michigan and New York, demonstrated significantly higher complication rates: pneumothorax rate was 15% (95% CI: 14.0-16.0%) and 6.6% of all biopsies (95% CI: 6-7.2%) required chest tube insertion. Furthermore, the population most likely to have a PPL requiring investigation (60-69 years old smokers, those with COPD) was also the most likely to suffer from procedural complications. 1% of procedures were associated with hemorrhage with 17.8% of this required blood transfusion.

Conclusion: PPLs

Guided bronchoscopic methods (EBUS GS/RP, ENB, VB/UB) have higher diagnostic sensitivity than TBLBx, but slightly lower sensitivity than CT-TTNA. the biggest advantage with a bronchoscopic approach is the lower complication rate; the diagnostic yield of each guided bronchoscopic technique is similar (42). Each technique has advantages and disadvantages and will ultimately depend on availability, local expertise, and lesion location. Only a large scale multicentre randomised trial directly comparing guided bronchoscopy to CT-TTNA will verify the merits of each procedure and determine when each procedure should be used. The weakness of contemporary data is that they are case series of non-consecutive patients where the selection criteria are not explicitly outlined and comparator groups are not used. Even when comparator studies are performed,

the results are dependent on operator expertise so that data are not necessarily generalisable.

Peribronchial/peritracheal/mediastinal lesions

Convex probe EBUS guided transbronchial needle aspiration (CP-EBUS TBNA)

Peribronchial and mediastinal lesions are accessible by conventional TBNA (cTBNA) however intimate anatomical knowledge is required to ensure safety and adequate diagnostic yield. Accurate assessment of an abnormal mediastinum is vital in lung cancer staging to guide best treatment. For several years, the gold standard for mediastinal staging has been surgical mediastinoscopy however this requires significant cost including hospital admission, general anaesthesia, and has associated morbidity and mortality (43). CP-EBUS TBNA addresses many of the shortcomings of cTBNA and surgical mediastinoscopy. Its rapid widespread adoption is due to its excellent utility, ease of use, ability to perform as a day case under light anaesthesia, and excellent patient satisfaction (44). Furthermore, the most recent American College of Chest Physician guidelines regarding staging of lung cancer recommend using EBUS TBNA, EUS FNA, or a combined approach over surgical staging as the best first test for investigating radiologically suspicious mediastinal lymph nodes (grade 1C) (45).

An integrated CP-EBUS TBNA scope/aspirating needle combination allows ultrasonic, real-time visualization of the needle inside the target lesion. The dedicated scope has a 6.9 mm outer diameter and 2 mm instrument channel whose distal end houses a CP-EBUS with flex of 120 degrees upward to 90 degrees downward. An inflatable balloon is sometimes applied over the probe to improve ultrasound signal, particularly in regions where the probe cannot be flexed against the bronchus wall. Vision is through a 30 degree oblique forward viewing fibre-optic lens with an 80 degree viewing angle. The dedicated 21 or 22 G needle is advanced to the distal end of the working channel and secured proximally onto the bronchoscope. Once the target is identified ultrasonically and doppler excludes overlying vessels, the needle is plunged into the lesion. The central stylet is moved back and forth to clear bronchial debris, and 8 to 10 aspirations are taken.

Several studies have proven the utility of CP-EBUS TBNA for mediastinal staging of lung cancer. In one study 163 lymph nodes were sampled in 105 patients.

CP-EBUS TBNA correctly predicted lymph node stage with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of 94.6%, 100%, 100%, 89.5%, and 96.3% respectively (46). A series of CP-EBUS TBNA of 572 lymph nodes from 502 patients demonstrated a sensitivity, specificity, and PPV were 94%, 100%, and 100% respectively with surgical staging as the gold standard (47). The same group performed CP-EBUS TBNA on NSCLC patients with a radiologically normal mediastinum, before undergoing surgical staging. For the detection of malignancy, sensitivity, specificity, and NPV was 92.3%, 100%, and 96.3% respectively, with no complications; performing CP-EBUS TBNA on NSCLC patients with a radiologically normal mediastinum avoided unnecessary surgical exploration in one of six patients (48).

Two large systematic reviews/meta-analyses confirm these findings and cement the utility of CP-EBUS TBNA in the staging of lung cancer. In the first (20 studies), sensitivity ranged between 85-100% and NPV ranged from 11-97.4%, with no serious complications reported (49). In the second (11 studies, P=1,299), sensitivity and specificity was 0.93 and 1.00 respectively; For the subgroup selected based on CT or PET findings sensitivity increased to 0.94 compared to only 0.76 without CT or PET. Only two patients had complications (0.15%) (50).

Yasufuku *et al.* performed one of the few prospective studies directly comparing CP-EBUS TBNA to mediastinoscopy for staging of lung cancer (51). Patients with confirmed or suspected NSCLC requiring mediastinoscopy as part of their staging investigations were eligible for inclusion. 153 patients underwent CP-EBUS TBNA followed by mediastinoscopy, with the operator blinded to the rapid on-site examination results; If lymph node involvement was present, patients proceeded onto surgical resection and these results were used as gold standard. Sensitivity, NPV and diagnostic accuracy for CP-EBUS TBNA and mediastinoscopy was 81%, 91%, 93% and 79%, 90%, 93% respectively, with no significant differences between the two in yielding true pathologic N stage (P=0.78).

Combined mediastinal staging with CP-EBUS TBNA and EUS-FNA (so called "medical staging") provides more complete staging allowing additional access to stations 8 and 9. Medical staging seems superior to either staging method alone (52-54). A randomized controlled study showed that medical staging had a higher sensitivity for nodal metastases and was associated with fewer unnecessary thoracotomies

when compared to mediastinoscopy (55). Both EUS-FNA and CP-EBUS TBNA can be performed in the one sitting using a single bronchoscope without compromising efficacy, and presumably saving time and money (56,57).

CP-EBUS TBNA equipment, technique and specimen preparation are critical. Diagnostic yield does not differ between 21 and 22 G aspirating needles, but the former results in fewer needle passes (58), better preserves histological structure, with the trade-off being more blood contamination (59). Although suction is traditionally applied to aspirate samples, a randomised controlled trial found no difference in specimen adequacy, quality or diagnosis between specimens with and without suction (60). Three aspiration passes per lymph node is diagnostically optimal with additional passes offering minimal benefit when no rapid onsite evaluation (ROSE) is available (61). The "tissue coagulum clot" method, which involves pushing the specimen onto a pre-cut piece of filter paper with the needle moved in a circular motion to build a cone shaped coagulum of clot and tissue, may increase amounts of diagnostic material when compared to conventional saline needle rinse (62). The use of mini-forceps-transbronchial needle forceps with a beveled end to facilitate penetration through the bronchus wall and jaws that can be opened under ultrasound guidance-is an alternate method to achieve larger volume samples and pilot studies confirm its safety and efficacy (63).

Histological subtyping from CP-EBUS TBNA reflects true histology. In one retrospective study, 88 patients who underwent CP-EBUS TBNA had these results compared to core biopsies and/or follow up surgery. Sensitivity, specificity, PPV, and NPV for CP-EBUS TBNA were 85%, 100%, 100%, and 89.7% respectively (64). A more recent study of 92 patients with NSCLC demonstrated a 76% agreement between needle aspirate and biopsy for subtyping (kappa =0.52), with agreement increasing if cell blocks were available (96%, kappa 0.91 *vs.* 69%, kappa 0.39 respectively) (65).

Mutational analysis is also possible from CP-EBUS TBNA samples. 154 out of 156 cases were successfully analysed (98.7%) for EGFR mutations using the PCR clamp technique on cell-pellets derived from needle-washed solution (66). Garcia-Olive and colleagues showed EGFR analysis was possible in 72.2% of patients undergoing CP-EBUS TBNA with metastatic nodal specimens (67). In a different study analysis for EGFR and KRAS sequences using COLD-PCR was achieved in 95.5% and 98.4% respectively of samples (68). A UK group found that 88% of their CP-EBUS TBNA samples were adequate for

mutational analysis using the Scorpion ARMS kit (69).

The actual false negative rate for CP-EBUS TBNA is a matter of debate; whilst specificity is unequivocally acceptable, sensitivity and NPV is more important when staging cancer. In 109 patients who underwent CP-EBUS TBNA of PET-avid N2 and N3 lymph nodes, 32 patients were tumour negative by CP-EBUS TBNA but subsequent biopsy in 19 showed malignancy in 7, four due to sampling error, and three due to detection error (70). Thus, in the setting of a high pre-test probability of nodal metastasis and no malignant cells on CP-EBUS TBNA, surgical biopsy should be used for confirmation (71).

Aside from mediastinal staging for NSCLC, CP-EBUS TBNA can diagnose central parenchymal lesions not visible on WLB, avoiding procedures associated with higher complication rates such as CT guided TTNA or mediastinoscopy (72-74).

Sarcoidosis can be reliably detected on CP-EBUS TBNA specimens. The combination of CP-EBUS TBNA and ROSE has high diagnostic accuracy, good interobserver agreement, and can inform the bronchoscopist of whether additional passes are necessary (75). Diagnostic accuracy of CP-EBUS TBNA is significantly higher than transbronchial biopsy or bronchoalveolar lavage (91.4% CP-EBUS TBNA, 65.7% BAL, 40% TBLBx) in Stage I sarcoid disease, although the three modalities have equivalent diagnostic rates in Stage II sarcoid disease (76). A systematic review and meta-analysis of CP-EBUS TBNA for sarcoidosis (15 studies, n=533) found a pooled diagnostic accuracy of 79% with only five minor complications reported, however significant study heterogeneity and publication bias were identified (77).

The diagnosis of sarcoidosis on CP-EBUS TBNA, however, should be tempered by the patient's pre-test probability of having this condition; in a case series of 1,275 patients undergoing CP-EBUS TBNA, granulomatous inflammation was found in 154 (12.1%) patients of whom 12 (7.8%) had a concurrent diagnosis of cancer, although no patient had both granulomatous inflammation and malignancy within the same lymph node (78). Patients with a high pre-test probability of malignancy but only granulomatous inflammation from CP-EBUS TBNA samples should be considered for additional sampling or close radiological follow up to ensure a benignity (79).

Cost effectiveness

Economic analyses validate the economic viability of CP-EBUS TBNA compared to cTBNA and mediastinoscopic

staging. In a retrospective cohort of 294 patients with thoracic lymphadenopathy from a University Hospital, 37 patients underwent cTBNA and 257 had CP-EBUS TBNA. 90% of the CP-EBUS TBNA group was diagnostic compared to 62.2% of the cTBNA group; a higher proportion in the cTBNA group needed additional surgical procedures such as mediastinoscopy, video-assisted thoracic surgery (VATS), or an open thoracotomy. The mean savings with CP-EBUS TBNA was $1,071.09 per patient (80). Improved cost efficacy was also found in a health technology assessment involving hospitals from the United Kingdom, Belgium, and the Netherlands (81). Study patients were randomized to either surgical staging alone, or CP-EBUS TBNA/EUS-FNA followed by surgical staging if negative. The 6-month cost of the former group was £10,459 per patient compared to £9,713 per patient with the latter approach, a saving of £746 per patient mainly through reducing mediastinoscopies and unnecessary thoracotomies.

Learning curve/training

Proficiency in CP-EBUS TBNA improves with experience, however the number of procedures required for proficiency is uncertain (82,83). A cusum (cumulative sum control chart) analysis determined that learning curve duration was highly variable, even for experienced bronchoscopists, with one operator almost immediately gaining competence, whilst another still on the learning curve after 100 procedures (84).

Trainees performing CP-EBUS TBNA increase procedure time, amount of sedation used, and complication rates (85). Obtaining proficiency using an CP-EBUS TBNA simulator before performing real procedures may address some of these issues; In one study, simulator training was equivalent to 15-25 "on-the-job" procedures in terms of procedure time and percentage of lymph nodes successfully identified (86,87). With the number of trainees wanting to acquire this new skill, coupled with the increasing focus on efficiency and reduction in complications, the EBUS simulator may become an increasingly valuable asset.

ROSE

ROSE of needle aspirates is thought to be beneficial in CP-EBUS TBNA but results have been varied. Potential advantages of ROSE for CP-EBUS TBNA include: quicker diagnosis, shorter procedure time, fewer needle passes per lymph node, and as a consequence reduction in complications.

In a prospective study, 120 patients suspected of having lung cancer with mediastinal adenopathy ≥10 mm were randomized to CP-EBUS TBNA with or without ROSE. In the ROSE group, the decision to make additional passes/procedures was based on ROSE findings at the operator's discretion; In the non-ROSE group, the target lesion underwent a minimum of three punctures, and additional punctures or bronchoscopic procedures were performed if the examiner deemed it necessary. There were significantly fewer punctures of the target lesion in the ROSE group (mean 2.2 punctures *vs.* 3.1 punctures, P<0.001) and significantly greater additional procedures in the non-ROSE group (57% non-ROSE *vs.* 11% ROSE). The mean bronchoscopy time, sensitivity and diagnostic accuracy did not differ between the groups.

ROSE results, however, need to be interpreted with caution as false negatives can occur (88) and concordance between staging and final pathological diagnosis is not perfect. On-site adequacy criteria have been proposed to reduce the risk of false negative specimens (89) but have not been prospectively validated.

CP-EBUS TBNA complications

CP-EBUS TBNA is a very safe procedure. Data from the prospectively enrolled American College of Chest Physicians Quality Improvement Registry, Evaluation, and Education (AQuIRE database) included 1,317 patients from 6 hospitals who underwent CP-EBUS TBNA for lymph node sampling (90). 19 patients (1.44%) had a complication with one patient dying from bleeding. Only TBLBx was associated with increased risk on multivariate analysis. Pneumothorax occurred in seven patients and TBLBx was the only variable associated with increased risk; [2.7% TBLBx had pneumothorax *vs.* 0.2% of those who did not (P=0.001)]. Factors associated with escalation in care included age >70, deep sedation or general anaesthesia, and inpatient status. There were no differences in complication rates between hospitals and outcomes were not associated with procedural volume. ROSE reduced the rate of subsequent TBLBx (P=0.006).

A questionnaire about CP-EBUS TBNA sent to 520 Japan Society for Respiratory Endoscopy-accredited facilities aimed to determine the rate of complications in Japan (40). Of 7,345 CP-EBUS TBNA performed in 210 facilities, 90 complications occurred (1.23%) with hemorrhage being the most frequent complication (0.68%), followed by infection (0.19%) and pneumothorax (0.03%). Only one death occurred (0.01%), the cause of which was cerebral infarction. Equipment related complications were

common, with breakage of the ultrasound bronchoscope and puncture needle in 1.33% and 0.2% respectively. These rates are slightly greater than those reported in two meta-analyses (0% and 0.15%) (49,50). Individual case reports detail infectious complications (91-96), needle breakage (97), intramural hematoma (98), and pneumothorax.

Conclusion: peribronchial/peritracheal lesions including mediastinal lymphadenopathy

CP-EBUS TBNA has revolutionised mediastinal staging of lung cancer with sensitivity approaching mediastinoscopy associated with few complications. The widespread adoption of CP-EBUS TBNA internationally by surgeons and physicians is a tribute to its utility, usefulness, simplicity and safety; It is one of the few diagnostic techniques that has truly revolutionized lung cancer diagnostics.

Conclusions

Innovative bronchoscopic techniques are allowing lung cancer to be diagnosed earlier and more accurately in an increasingly non-invasive fashion. Peripheral lesions are targeted by RP-EBUS/GS, ENB, and UB/VB; mucosal lesions can be identified with NBI and AFB; OCT allows cellular analysis without the need for biopsy; and central peribronchial lesions can be accurately localized and sampled with EBUS TBNA. Whilst the role of established techniques like RP-EBUS/GS, CP-EBUS TBNA, NBI and AFB are well established, data regarding emerging techniques such as ENB and OCT are immature and require further study to establish their utility.

The field of interventional diagnostic pulmonology is rapidly advancing, with the aim of safer, less invasive and more accurate modalities to identify and diagnose lung cancer earlier. Coupled with newer therapeutics such as stereotactic body radiation and targeted therapeutic agents, it is hoped that lung cancer mortality will no longer be the most common cause of cancer related mortality worldwide.

Acknowledgements

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Endoscopic intervention of lower airway foreign matter in adults— a different perspective

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Background: Lower airway foreign matter (FM) is uncommonly encountered in adults. This study reviews FM in the lower airway that required bronchoscopic intervention.
Methods: A retrospective review of patients with FM in the lower airway seen at a tertiary hospital between 1996 and 2014 was undertaken.
Results: Lower airway FM was removed in 80 out of 18,650 bronchoscopies performed. Forty-seven were males, with mean age of 50.8 (range, 29-78) years and mean symptom duration of 10.3 months (range, 1 day -20 years). The most common symptoms were persistent cough, followed by dyspnea, hemoptysis, episodes of choking and fever. Three-quarters of the patients had risk factors of either aspiration or iatrogenic cause for FM in the airway. FM identified following bronchoscopy was classified as: organic (31.3%), inorganic (46.3%) and endogenous matter (22.4%). Iatrogenic etiology was evident in four-fifths of the patients with inorganic FM (stents being the most common). Forty-eight (60.0%) patients had FM removed via flexible bronchoscopy, and the remainder via rigid bronchoscopy. The majority (27 out of 32) of FM removed by rigid bronchoscopy could not be removed using the flexible scope. This was primarily due to retrieval of stents [24] could only be done with the rigid bronchoscope. There were four FM-related complications (three bronchostenosis, one actinomycosis).
Conclusions: There is an increasing indication for bronchoscopists to retrieve FM, particularly of iatrogenic and endogenous sources, lodging in the lower airway of adults. There may be a reversing trend in the utilization of rigid bronchoscopy, mainly due to the increasing need to remove airway stents as more are deployed.

Keywords: Bronchoscopy; foreign bodies (FB); stents; complications; techniques

Introduction

Lower respiratory tract foreign bodies (FB) are more commonly encountered (75-85% of all cases) in children than in adults, with incidences peaking in those under 3 years of age (1,2). It is rare in the latter, with an estimated incidence of 0.2% to 0.33% of all bronchoscopies performed (3-5). However, bronchoscopists have encountered increasing need to remove foreign matter (FM) entering and lodging in the lower airway. This study reviews the classification of lower airway FM, emerging trends of treatment in a tertiary academic hospital, and the implication with regards to the differences in intervention compared to published literature on lower airway FB in adults.

Methods and materials

A retrospective review of patients with FM in the lower respiratory tract seen at a tertiary hospital between 1996 and 2014 was conducted following approval from the Ethics Committee and Hospital Institutional Review Board (number 2013/860/C). Hospital records including

Table 1 Clinical and radiological profile of adults who required the removal of lower airway FM

Clinical features	Total N=80
Age	50.8 (range, 29-78) years
Male:female	47:33=1.4:1
Duration of symptoms	5.3 months (range, 1 day to 20 years)
Symptoms	Cough, 60 (75%) Dyspnea, 23 (28.8%) Hemoptysis, 17 (21.3%) Choking, 10 (12.5%) Fever, 9 (11.3%)
Respiratory failure	8 (10%)
Radiographic findings	Normal, 24 (30%) Alveolar infiltrates, 16 (20%) Lung collapse/atelectasis, 18 (11.4%) Opaque foreign body, 27* (33.8%) Hyperinflation, 1 (1.3%)

* including 24 stents. FM, foreign matter.

Table 2 Classification of lower airway FM

Source/type, total N=80 [%]	Risks, n [%]	Specific FMs
Exogenous/organic matter, 25 [31.3]	Aspiration, 15 [18.8]	Chicken bone, 3 Fish bone, 2 Nuts, popcorn, seeds, 5 Vegetables, 3 Apple, 2 Fish ball, 1 Unspecified food, 9
Exogenous/inorganic matter, 37 [46.3]	Aspiration, 2 [2.5]	Pill, 2 Talc powder, 1 Pin, 1 Metallic fragment, 1 Thumbtack, 1 Spoon handle, 1*
	Iatrogenic, 30 [37.5]	Stents, 24* Sutures, 4* Voice prosthesis, 2
Endogenous matter, 18 [22.4]	Aspiration, 12 [15]	Bronchiolithiasis, 1 Jaw bone, 1* Tooth, 2 Blood clots, 4** Mucus plugs, 10**

*, all stents, 2 sutures, 1 fragment of jaw bone could only be removed with the rigid bronchoscope; **, all with partial or complete lung collapse. FM, foreign matter.

clinical, radiological, procedural and pathological charts of patients with FM from the trachea to the lungs were studied. FM is defined as any matter that has entered and lodged in the lower airway, requiring its removal by bronchoscopic intervention. Blood clots and mucus plugs that did not result in partial or complete lung collapse were excluded. The flexible bronchoscope (Olympus, Japan) was intubated either orally or via the endotracheal tube performed under topical anesthesia and moderate sedation with intravenous midazolam or propofol and fentanyl. Rigid bronchoscopy (using Bryan-Dumon bronchoscope, Bryan Corporation, USA) was performed under deep intravenous sedation allowing spontaneous respiration and assisted manual ventilation. The flexible bronchoscope was used through the rigid tube in all cases of rigid bronchoscopy. The former allowed both accessibility to the distal airway and thorough evaluation for residual FM fragment. All categorical variables are reported as counts and percentages and continuous variables are reported as means and ranges.

Results

Clinical presentation

Eighty patients (0.43%) of a total of 18,650 bronchoscopies performed over 18.5 years were identified to have lower airway FM that required intervention, *Table 1*. Four (5.0%) patients were treated for recurrent respiratory tract infections over 4 months to 20 years before the diagnosis of airway FM. Three-quarters of the patients had risk factors of either aspiration or iatrogenic cause for FM in the airway, *Table 2*. Risks for aspiration were neuromuscular dysphagia [23], encephalopathy [5], and severe facial trauma [1] from vehicular accident 20 years prior.

Investigations

Chest X-rays (CXR) were normal (30%) or showed presence of FM (33.8%, *Figure 1A*) in two-thirds of the patients. Chest computed tomography (CT) scans were performed in 35% of patients and provided more information that contributed to the planning of airway intervention (*Figures 1-3*).

Identification of all FM was made following bronchoscopy; these can be classified into three groups, *Table 2*: exogenous organic (31.3%), exogenous inorganic (46.3%) and endogenous (22.4%) matter. The risk for aspiration was prevalent in about two-thirds of the patients who had either organic or endogenous matters in the
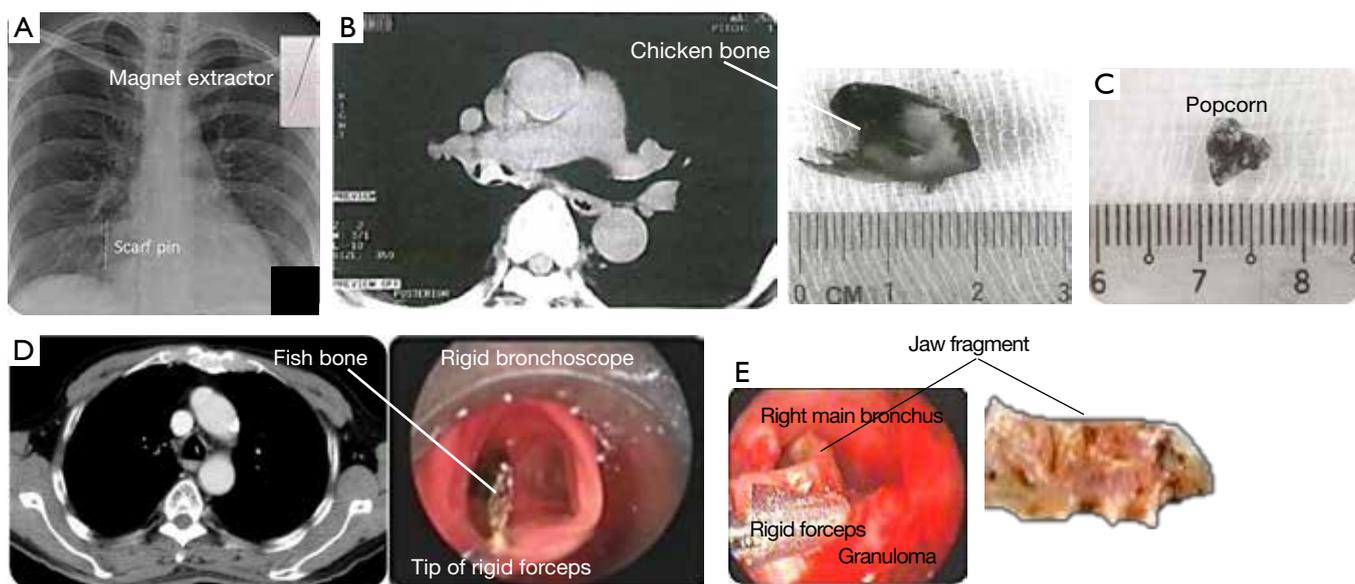
Figure 1 Organic foreign matter (FM) shown in: Inorganic FM shown in: (A) a headscarf pin held between the lips of a young lady aspirated during sneezing evident on chest X-ray. A magnet extractor was used to retrieve the pin out of the medial basal segment of the right lower lobe; (B) chicken bone in the right main bronchus seen on CT scan image and upon removal and (C) popcorn removed from left lower lobe bronchus; (D) CT scan and rigid bronchoscopy findings at the lower trachea showing the top of a fish bone aspirated 9 months prior; Endogenous FM shown in: (E) 4 by 3 cm fragment of the jaw aspirated by a middle aged subject during a car accident a couple of decades ago. Removal was possible only with the rigid forceps via the rigid bronchoscope.

airway. Iatrogenic etiology was evident in four-fifths [30] of the patients with inorganic matter of which stents [24] were the most common FM that required removal most often from tuberculous strictures [10], *Tables 2,3*. A total of 24 (7.0%) out of 341 stents deployed during the study period required removal. About two-thirds [15] of the stents were retrieved as they were no longer needed to maintain airway patency and the rest [9] were removed due to complications (6 stent granulomas and 3 stent migration). All stents removed were silicone stents except for four which were metallic (ultraflex) stents (*Figures 2,3*) (6).

Intervention and outcome

Forty-eight (60.0%) patients had FM removed via flexible bronchoscopy, with the remainder via rigid bronchoscopy. The majority of FM removed by rigid bronchoscopy (27 out of 32) could not otherwise be done with the flexible bronchoscope (*Figures 1E,2,3*) (6). This was primarily due to the fact that stent retrieval (in 24 of the cases) was only possible with the rigid bronchoscope. Accessory tools utilized were: grasping or biopsy forceps [51], suction [27],

neodymium-doped yttrium aluminium garnet (Nd-YAG) laser [6], urokinase (2 involving blood clots) and one each for cryoapplication (removal of mucus plug), Dormia basket (tooth) and magnet extractor (pin) (*Figure 1A*). One patient had severe airway inflammation and stricture due to inhalation of cosmetic talc diagnosed on histological and spectral analysis of bronchial biopsy; the patient responded well to a course of oral steroids and avoidance of talc exposure. Complications during bronchoscopic retrieval of FM were: transient hypoxia [20], bleeding from granulomas (4; easily controlled with ice-cooled saline flush or Nd-YAG laser application) and severe mucosal damage from removal of metallic stents [4] (*Figure 3*) (6). All but four patients recovered completely from the effects of FM: two patients had significant residual airway fibrotic strictures following removal of metallic stents, one who aspirated two tablets of Domperidone (*Figure 4*) had complete stenosis due to fibrosis and severe malacia of the right bronchus intermedius despite repeated interval balloon dilatation, and the last patient had severe localized bronchiectasis requiring lobectomy two years after removal of a jaw fragment (*Figure 1E*) as a result of untreated airway actinomycosis.

Figure 2 Marked upper trachea stricture secondary to granulomas growth through the bare proximal 7.5 mm of the covered ultraflex stent that was deployed in a young subject with tracheal stenosis caused by tuberculosis. The patient was referred for removal of the stent which was possible only using the rigid forceps via the rigid bronchoscope, see *Figure 3*.

Video 1. Endoscopic intervention of lower airway foreign matter (FM) in adults—a different perspective

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Figure 3 Endoscopic intervention of lower airway foreign matter (FM) in adults—a different perspective (6).
Available online: http://www.asvide.com/articles/703

Discussion

Foreign body (FB) aspiration occurs most commonly in toddlers when they explore things by putting them in their mouths (1,2). In adults, FB aspiration is uncommon and is caused mostly by the failure of airway protective mechanisms. Previous studies have classified airway FB into organic and inorganic objects (2,4,7,8). In this study, the term FM is preferred as it encompasses any matter that has entered and lodged in the lower airway from a source that maybe exogenous or endogenous to the body. This includes luminal matter requiring bronchoscopic intervention for removal, such as deployed airway stents, large blood clots and mucus plugs. Therefore, both the risk factors (74.0%) and the incidence (0.43%) of lower airway FM in adults reported here are higher than the respective figures reported by Ramos et al (44.0%) (4) and Debeljak *et al.*

(0.2%) (3). A higher incidence of lower airway FM (0.33%) has been reported in studies (4,5) subsequent to Debeljak *et al.* The results here are attributed to the study being conducted in a thoracic surgery referral centre (4) and 90% of the cohort being at high risk for aspiration (5). Common organic materials inadvertently aspirated, are bones, nuts, peas, beans, seeds, vegetables and apple (4,5,9) Bone is the commonest FB retrieved in most studies (3,5,9,10) The predominance of aspirated matter has been correlated to cultural and social factors such as eating habits, customs and climate (5,7,9,10) The etiology of inorganic airway FM can either be inhalational or iatrogenic. Small objects that are placed at or in the mouths, including toothpicks, nails, screws, needles, pins, clips, caps of writing stationery and dental parts; can be unintentionally inhaled into the lower airway during sneezing, coughing or laughing. These actions require sudden deep inspiration. Al-Sarraf *et al.* (7) reported headscarf pin tracheobronchial aspiration as a distinct clinical entity in females who placed the pins in their mouths prior to securing their veils. As these pins are radiopaque (*Figure 1A*), the diagnosis is generally made earlier than for organic FB aspiration. Lower airway FM from iatrogenic source is invariably inorganic matter and has been related to tracheostomies, voice prostheses, as well as dental and bronchoscopic procedures (3,4). In this report, iatrogenic inorganic matters include sutures and voice prostheses in the first 5 years and airway stents in the subsequent years of the study period [1996-2014]. Airway stenting has become an important therapeutic option in many mechanical obstructive airway lesions. The use of more stents in the airway will concomitantly require increased removal. In this study, 7.0% of deployed stents were removed, often from benign airway lesions and also because they are no longer functional or needed in maintaining airway patency rather than as a result of stent complications. Endogenous matter lodged in the lower airway can enter the lumen either from the patient's oral cavity (e.g., fractured facial bones due to trauma and dislocated teeth) or from the lower respiratory tract (e.g., large clots, mucus plugs, bronchiolitis) (3,10,11).

As tracheobronchial FB is uncommon and clinico-radiological presentation is nonspecific, a high index of suspicion is required (10). A history suggestive of FB aspiration is reported in up to 42% of cases and the longest retained lower airway FB before removal was 40 years (9). These FBs can result in airway obstruction via a direct physical mechanical effect and/or an indirect chemical/reactive effect, causing severe acute mucosal inflammation, granulation tissue

Table 3 Characteristics of patients who required removal of lower airway stents

Primary diagnosis, N=24	Mean duration of deployed stent (months)	Indication for removal (all via rigid bronchoscopy)
Tuberculosis, 10	18	No longer needed, 7
		Stent (metallic) granulomas, 3
Post-transplant anastomotic site stenosis, 1	8	Stent (metallic) granulomas, 1
Post intubation stenosis, 3	14	No longer needed, 3
Malignant tumors, 7	5	No longer needed, 4
		(2 had surgery, 2 responded to chemotherapy)
		Stent migration, 3
Bronchopleural fistula, 1	6	No longer needed, 1
Tracheobronchomalacia, 2	11	Stent (silicone) granulomas, 2

Figure 4 A middle aged subject aspirated 2 domperidone tablets lodged in the right main bronchus and bronchus intermedius (A,B) evident on CT scans but normal CXR. She had dysphagia due to the effects of radiotherapy for nasopharyngeal carcinoma 12 years prior. These pills were removed using the Dormia basket via flexible bronchoscope through the endotracheal tube under general anesthesia. However, the intense chemical injury resulted in complete fibrotic stenosis of the bronchus intermedius evident on bronchoscopy 8 weeks later (C). CT, computed tomography; CXR, chest X-rays.

and airway stricture which may eventually be irreversible due to fibrotic scarring and malacia. Some of these FBs, such as pills (12,13), inhalational particles (14) and vegetative matters induce chemical reactions as they disintegrate, result in severe mucosal inflammation and formation of granulation tissue within few hours following inhalation. Vegetative matters such as beans, seeds and corn can absorb water and on subsequent swelling, change partial obstruction to total, leading to atelectasis or complete lung collapse. Infection, including actinomycosis (9,15,16), endobronchitis and bronchiectasis can then ensue. Destructive irreversible bronchiectasis may develop many years after unrecognised aspiration of a FB. Post-obstructive sequelae include subcutaneous emphysema, pneumonia, abscess and empyema. One of the largest and most

recent series of cases reviewed by Dong *et al.* (9) attributed the high incidence (79.5% of 200 cases) of FB-related complications to the delayed diagnosis by more than one month in 58% of the patients. Therefore, it is crucial to remove these FB, especially chemical matter, as soon as possible before more complications set in and retrieval becomes technically more difficult and unsafe once the FB embeds in the bronchial wall, particularly, when 'engulfed' by exuberant granulation tissue (5,9).

Diagnostic evaluation comprises of imaging and bronchoscopy. In the series reported by Al-Sarraf *et al.* (7), all aspirated headscarf pins were evident on CXR, leading directly to therapeutic bronchoscopy in the majority of cases. A third of the patients (33.8%) in this study had visible FB

on CXR (with the majority of these being stents). However, about the same proportion of patients had normal CXR (30%). The CXR may remain normal in the first 24 hours and beyond in 15-25% of the cases reported (1,3-5,10,13). The absence of a radiopaque FB does not exclude the possibility of FB aspiration, even if it is a metallic object as only 7-21% of aspirated FB (in particular organic matter) is radiolucent (1,3,5,8,10). A standard radiological work-up including soft tissue neck radiograph, posteroanterior and lateral CXR should be performed in the initial diagnostic evaluation as it provides data that may lead to further imaging with CT scan and/or diagnostic bronchoscopy in the majority of cases (1,3-5). The presence of atelectasis, lung collapse, pulmonary infiltrates, bronchiectasis, air trapping and mediastinal shift on the chest radiographs may be suggestive of FB aspiration. The radiological finding of unilateral or segmental hyperaeration, due to ball-valve effect of the FB lodged in the airway, can be better seen on expiratory radiographs. Chest CT scan provides further information and aids in the planning of intervention (*Figures 1-3*). Bronchoscopy is frequently required for the diagnosis to be certain. When FB aspiration is not suspected clinically or the FB is absent on bronchoscopy (such as a dissolved pill), the pathologist may play an important role in making the diagnosis (12,13,17). Histological identification of the aspirated material may then be necessary for a definitive diagnosis. As an example, histological and spectral analysis of the bronchial biopsy of one patient in this study confirmed the diagnosis of inhaled cosmetic talc as the cause of bilateral severe bronchial inflammation and stricture.

In 1897, Gustav Killian performed the first bronchoscopy by removing a bone in the airway through a rigid tube (18). With clinical application of flexible bronchoscopy in 1967, the flexible bronchoscope has been the preferred instrument for the diagnosis and the removal of lower airway FB in adults (3,5,9,10,19). The rigid bronchoscope plays a complementary role to the flexible scope and is occasionally an essential therapeutic tool as the chronicity, size, and nature of the FM (*Figure 1E*) can render its retrieval unsafe and/or near impossible with the flexible scope (3,4,7,9). Rigid bronchoscopy, rarely followed by surgery, should then be therapeutic options (4,8,10). Despite the majority of published studies reporting the successful removal of FB with the flexible bronchoscope (at a rate of 91-97%) (3,5,9,19), this study indicated a relatively higher frequency of the rigid bronchoscope (40%) as the therapeutic instrument. This is because it is preferred to the flexible bronchoscope for the removal of large and/or sharp

objects, with the majority (27 out of 32 cases reviewed) of FM removed by rigid bronchoscopy not removable using the flexible bronchoscope. It is worth noting that the retrieval of all stents [24] was only possible with the rigid bronchoscope. Silicone stents can be easily removed, but the retrieval of metallic stents deployed for more than 6 weeks is difficult and frequently results in severe mucosa injury (*Figure 3*) (6) with an attendant risk of transmural rupture. Due to the accessibility of rigid bronchoscopy in the study hospital, it is the preferred option when there are clear advantages such as the easier and/or safer retrieval of the FM.

Bronchoscopic intervention is primarily aimed at the removal of intraluminal lesions employing various bronchoscopic techniques. A range of accessory devices is required to retrieve FM with different characteristics in size, shape, edge, texture, consistency, and state of matter. Tools and procedures reported to be useful are grasping or biopsy forceps, wire baskets, Fogarty balloons (used to separate wired stents from mucosa), cryotherapy, Nd-YAG lasers, argon plasma coagulators, electrocautery, suction catheters, magnet extractors, snare loop catheters and fluoroscopy (1,3,4,7,9,10). The Nd-YAG laser is a versatile accessory to use, as it can ablate granulomas and release embedded FM, secure hemostasis and resect large solid matter allowing for their piecemeal removal. The retrieval of FM using the flexible bronchoscope through an endotracheal tube has the advantage of easy direct access to the lower airway, especially when repeated extubation of the scope is required for piecemeal removal of the FM or cleaning of the scope lens (3,5). It is safer to grasp the sharp end of small object through the tube (7); the larger object can then be wedged against the tip of the endotracheal tube and removed en bloc with the scope. It is therefore less likely that the FM will be dislodged at the vocal cords, pharynx, mouth and nose. However, there has been a preference in removing large or sharp object with the rigid bronchoscope in this and previous studies (7,8).

The use of thrombolytic agents on airway clots has been previously documented (20). In the current study, urokinase was administered to a large clot in the main bronchus of two patients. Mucus and blood clots can result in acute airway occlusion leading to lung collapse and the removal of such semi-solid material (including food material) containing significant amounts of water with bronchoscopic suction can be challenging. The best therapeutic tool is cryoapplication on the water-containing matter (21). The cryoprobe operates using the Joule-Thomson effect,

in which a compressed gas is released at high flow and undergoes expansion. This rapidly cools the probe tip to near –80 ℃ within seconds and allows for the adhesion of the probe to any material containing water and retrieval of the frozen material. The advantage of the cryoprobe is that a large amount of material can be extracted during the freeze-thaw cycle.

Although none of the patients in this study required surgical removal of FM in the lower respiratory tract, it is conceivable that there will be rare instances for the need to intervene surgically (3,5,10) as the FM may be too distally or deeply embedded in the lower respiratory tract, rendering bronchoscopic removal impossible or unsafe.

Full and rapid recovery is expectant upon early mechanical removal of the FM (5,7,9,10). The cessation of inhalational exposure to minute particles and a course of steroids may suffice in rare instances. However, follow-up chest imaging and, if necessary, bronchoscopy is recommended to detect chronic complications (10,12,15). As illustrated for four patients in this study, irreversible airway destruction such as fibrotic strictures, bronchomalacia, bronchiectasis and actinomycosis are known complications even after removal of FM. Granulation is invariably present adjacent to long-standing retained FM. However, when there is exuberant tissue in the presence of aspiration of oropharyngeal content, actinomycosis must be considered and biopsy for microbiologic and histological studies should be done. Although a previous study (2) suggests resolution of granulation tissue and bronchiectasis after FB extraction, this may not occur in the presence of untreated coexisting airway actinomycosis. A prior course of antibiotics will reduced the granulation tissue, easing the removal of the FM. Conversely, the removal of FM must be followed by an adequate duration of antibiotics to avert lobectomy (10,15).

This study, like previous ones, suffers from the weakness associated with a retrospective study conducted over extended period, given that FM in the lower respiratory tract is indeed uncommon in adults. The evolving therapeutic tools developed over a long period of 18.5 years also result in the heterogeneity of lower-airway FM management. This study included FM as any matter that has entered and lodged in the lower airway requiring bronchoscopic intervention. Therefore the number of aspirated FBs [37] is much lower than reported in few larger studies (3,5,9). However, it provides the data on removal of perhaps the most common iatrogenic source of FM (stents) and endogenous matter including large obstructive clots and mucous plugs.

Conclusions

Early recognition and removal of lower airway FM is paramount in minimizing or averting complications such as airway strictures and infections. Lower airway FM encompasses not only aspirated FB but also from iatrogenic, the most common being stents for tuberculous strictures; and endogenous sources. There is an increasing indication for bronchoscopists to retrieve FM, particularly those of iatrogenic and endogenous sources, entering and lodging in the lower airway. There is likely a paradigm shift back to the use of the rigid bronchoscope in this setting. This is primarily due to the increasing need to remove airway stents with their increased deployment. When the rigid tube system is utilised more often, it will be made more accessible; this creates a tendency for the safer, easier and faster removal of FM, escalating the demand for rigid bronchoscopy. From the patients' perspective, rigid bronchoscopy confers increased comfort, as it is performed under deep sedation. The flexible bronchoscope through the rigid tube allows accessibility to the distal airway. The armamentarium of endoscopic accessory devices has widened and tools traditionally used for tumor ablation are useful adjuncts in the retrieval of FM. In particular, the relatively cheap and safe cryosystem will allow the removal of water-containing FM, including large airway clots and mucus plugs, with greater efficiency compared to bronchoscopic suction.

Acknowledgements

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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Indications and interventional options for non-resectable tracheal stenosis

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Abstract: Non-specific presentation and normal examination findings in early disease often result in tracheal obstruction being overlooked as a diagnosis until patients present acutely. Once diagnosed, surgical options should be considered, but often patient co-morbidity necessitates other interventional options. Non-resectable tracheal stenosis can be successfully managed by interventional bronchoscopy, with therapeutic options including airway dilatation, local tissue destruction and airway stenting. There are common aspects to the management of tracheal obstruction, tracheomalacia and tracheal fistulae. This paper reviews the pathogenesis, presentation, investigation and management of tracheal disease, with a focus on tracheal obstruction and the role of endotracheal intervention in management.

Keywords: Airway stent; laser therapy; rigid bronchoscopy; tracheal stenosis; large airway obstruction

Introduction

Large airway obstruction, particularly tracheal disease, is widely believed to be under-diagnosed and under-reported. Recognition of the precipitants to tracheal obstruction and of the common clinical manifestations and investigation findings will facilitate prompt and appropriate management. Rigid bronchoscopy is increasingly popular for the diagnosis and management of tracheal obstruction, and has a role in the delivery of interventional bronchoscopy. Surgical management is often definitive but patient selection and preparation is essential for surgical success.

This paper reviews the pathogenesis, presentation, investigation and management of tracheal disease, with a focus on tracheal obstruction and the role of endotracheal intervention in the management of non-resectable disease. It is intended that this paper will provide guidance for those involved in the care of patients with tracheal disease.

Tracheal anatomy and physiology

The trachea extends from the lower body of the cricoid cartilage to the carina and is normally between 110 and 130 mm in length. The tracheal diameter is typically up to 22 mm in males and up to 18 mm in females. Air is a fluid which flows down a pressure gradient, from higher to lower pressure. Airflow can be laminar or turbulent, with the flow rate defined as the amount of fluid moving per second. When flow is laminar, the airflow rate is directly proportional to the pressure gradient but when flow is turbulent, the airflow rate is proportional to the square root of the pressure gradient. Turbulence thus increases airflow resistance, necessitating a higher pressure difference to maintain a given flow rate. Reynolds number is a non-dimensional parameter that governs the change from laminar to turbulent air flow. It is calculated by multiplying mean fluid velocity, density and airway diameter then dividing by fluid viscosity. The diameter of the trachea affects airflow by this equation and any abrupt variation in tracheal diameter produces a transition point which also predisposes to turbulence.

Tracheal obstruction

Congenital or acquired tracheal narrowing may result from

intrinsic tracheal stenosis and/or external compression, at any anatomical level. Variation in the tracheal diameter alters the airflow dynamics in the direction of increased airflow resistance. Tracheal stenosis often results in varying degrees of distortion along the vertical plane in addition to airway narrowing. When the trachea becomes pulled away from or twisted within its normal anatomical path, this further predisposes to airway turbulence and increased resistance.

Congenital

Tracheal stenosis is rarely congenital but may result from posterior fusion of the tracheal rings. By contrast, congenital tracheal webs are well recognised, with 75% occurring at the level of the glottis. Tracheal webs differ from tracheal stenosis due to the absence of a cartilaginous framework. Both may present in adult life.

Congenital cardiovascular anomalies can result in extrinsic compression of the trachea. Most commonly, early bifurcation of the innominate artery compresses the anterior tracheal wall, appearing pulsatile at bronchoscopy. Other causes include anomalies of the subclavian artery and vascular rings, such as congenital double aortic arch, which encircle the trachea causing circumferential compression.

Acquired

Trauma

The cartilaginous trachea has a natural tendency to narrow and fibrose in the face of injury. Tracheal trauma is the most common cause of benign tracheal stenosis and is a feared complication of prolonged endotracheal intubation or tracheostomy tube placement. The presence of tracheal stenosis can necessitate re-intubation and delay respiratory weaning in intensive care unit patients or can present many years later.

The reported incidence of tracheal stenosis following endotracheal intubation ranges from 6-21% and following tracheostomy ranges from 0.6-21% (1-3). With the trend towards early tracheostomy as an aid to respiratory weaning, and increasing numbers of successful discharges from intensive care units, the incidence of tracheal complications is rising.

Stenosis occurs when pressure and friction on the mucosal surface stimulates inflammation and pressure necrosis. Granulation tissue formation is followed by fibroblast proliferation, scarring and contracture. Stenoses can develop after as little as 36 hours of endotracheal intubation but the risk of stenosis rises with duration of intubation. Most strictures occur at the site of the tube cuff, with reduced incidence following the introduction of compliant, large volume, low pressure cuffs (4).

After tracheostomy, stenosis most commonly occurs at the stomal site (3,5). Wound sepsis is a predisposing factor (6). Pre-existing chronic lung disease and airway infection are also associated with tracheal stenosis post endotracheal intubation or tracheostomy (7).

Trauma may also arise from thermal or chemical burns (including chemical warfare agents), resulting in localised stenosis.

Infection

Airway infection alone can result in the development of tracheal stenosis. Tuberculosis is the most common cause of post-infective stenosis but diphtheria, syphilis and fungal infection (e.g., histoplasmosis, blastomycosis) are also recognised causes.

Non-infectious inflammation

Non-infectious inflammatory conditions causing tracheal stenosis include collagen vascular disorders (e.g., Wegener's granulomatosis), sarcoidosis, amyloidosis and chronic atrophic polychondritis. Diffuse inflammatory and infective processes often result in multi-level tracheobronchial stenoses.

Neoplastic

Airway obstruction develops in 20-30% of lung cancer patients (8), however, tracheal compromise occurs in less than 1% of all malignancies (9). Direct tumour invasion of the trachea by a bronchogenic malignancy is more common than metastatic involvement of the trachea. Primary benign tumours of the trachea such as chondromas, fibromas, hemangiomas, and squamous papillomas are rare causes of tracheal stenosis. Extrinsic compression of the trachea can occur from malignant lymphadenopathy, thyroid and mediastinal tumours.

Iatrogenic

The insertion of a tracheal stent (e.g., for tracheobronchomalacia) can, paradoxically, lead to stenosis due to tracheal irritation and the formation of granulation tissue at either end of the stent. Cervico-mediastinal radiotherapy is another recognised cause of stenosis.

Other

Tracheopathia osteochondroplastica is a rare, but increasingly recognised condition in which there is the idiopathic development of focal or diffuse, osseous and/or cartilaginous nodules in the submucosa of the trachea and bronchial walls. The posterior membranous portions of the trachea are characteristically spared. Significant tracheal stenosis and/tracheomalacia can result.

Superior mediastinal pathology can cause extrinsic tracheal compression. Most frequently this arises from lymphadenopathy secondary to infection, inflammation or neoplasia, but abnormalities of the aortic arch such as dissection or aneurysm can also compress the trachea (10). Thyroid goitre may also cause extrinsic compression, particularly if there is retrosternal extension.

Idiopathic

Idiopathic tracheal stenosis is rare, representing 3-5% of cases. Most commonly these stenoses develop at the level of the cricoid cartilage and are restricted to young women (11). Pathologically there is extensive keloidal fibrosis and mucus glands dilation which may represent a form of fibromatosis (12).

Tracheal fistulae

Any aggressive tracheal pathology can disturb the integrity of the tracheal wall resulting in communication with the mediastinum. Iatrogenic, traumatic and malignant cases are the most prevalent. Infection as an aetiological factor (tuberculosis, HIV infection, mediastinitis) has reduced in recent years. Communication may also be established between the tracheobronchial tree and the oesophagus, resulting in tracheo-oesophageal (or bronchial-oesophageal) fistulae. Acquired tracheo-oesophageal fistulae are frequently the result of mediastinal malignancy. Tumours arising from the oesophagus, trachea, lungs, larynx, thyroid and lymph glands have all been reported to cause fistula formation. Tracheo-oesophageal fistulae can also be congenital. These typically present in the neonatal period but may rarely present in adulthood.

Tracheomalacia

Tracheomalacia is characterised by flaccidity of the tracheal cartilage, leading to airway collapse during expiration. The condition may extend to involve the bronchi (tracheobronchomalacia). Significant airway malacia is defined as a greater than 60% reduction in luminal diameter.

Congenital tracheomalacia results from a developmental defect in the cartilage of the tracheal wall. Tracheomalacia may also develop in the context of congenital conditions such as cystic fibrosis, Mounier-Kuhn syndrome, Marfan syndrome, Ehlers-Danlos syndrome, and congenital trachea-oesophageal fistulae.

Acquired tracheomalacia is associated with prolonged endotracheal intubation and tracheostomy, trauma, head and neck surgery, radiotherapy, and inflammatory conditions such as polychondritis. Intrinsic tracheal disease such as tracheal stenosis and previous tracheal stenting may also contribute to weakening of the airway support and malacia. When tracheomalacia occurs in the absence of a clear pathophysiology, these patients are often obese, with smoking related lung disease or recurrent/chronic airways infection.

Diagnosis of tracheal disease

The diagnosis of tracheal disease is a recognised challenge because of the broad range of aetiologies and the non-specific nature of presentation, which often has an insidious onset at first. Thus, a detailed patient history and examination is imperative to guide further investigation and management. Sometimes when the patient presents in extremis this is not possible or appropriate, and rapid intervention in a controlled environment with appropriately skilled personnel is central to a successful outcome.

History and examination

Symptoms depend on the location and degree of airway narrowing, additional airway distortion and concurrent thoracic pathology. Most commonly, patients report shortness of breath on exertion, which may progress to dyspnoea at rest. Symptoms occur on exertion when the tracheal diameter is significantly reduced to 8 mm (13). Cough and wheeze are common. Airway obstruction may lead to difficulty with sputum clearance and recurrent infection. The combination of exertional dyspnoea and wheeze is frequently mistaken for chronic bronchitis or asthma. Failure to respond to bronchodilators should not be overlooked.

It is not uncommon for patients with tracheal disease to present with acute respiratory distress, even in benign disease. These presentations are usually triggered by the partial or complete occlusion of the abnormal airway by sputum or
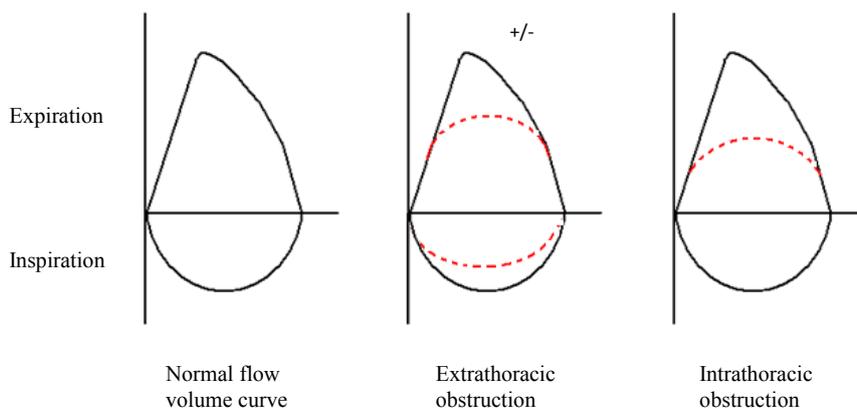
Figure 1 Flow volume curves showing upper airway obstruction

haemorrhage.

History taking should focus on potential tracheal insults such as intensive care admission, hoarseness after general anaesthesia (suggestive of traumatic injury), or respiratory tract infections. A full systems enquiry may also reveal information relevant to the underlying diagnosis.

Respiratory examination is often normal until there is severe tracheal stenosis or secondary airway occlusion due to sputum or haemorrhage. Stridor occurs when the tracheal diameter is less than 5 mm (13). Examination should explore the underlying diagnosis, looking carefully for signs such as a tracheostomy scar, goitre, lymphadenopathy or the classical nasal changes of Wegener's granulomatosis.

Investigation

When tracheal disease is suspected, first line investigations should include targeted blood tests to look for the underlying diagnosis (e.g., inflammatory markers, autoimmune screen), pulse oximetry and/or arterial blood gas analysis and standard chest radiography. These investigations are often normal.

Lung function testing

When spirometry results are interpreted correctly, ensuring technical requirements are met, they can be the first investigation to suggest the diagnosis of tracheal obstruction. Flow volume diagrams provide an indication of the severity of airflow obstruction and the location of airway obstruction, i.e., intrathoracic or extrathoracic (14).

The pressure surrounding the intrathoracic airway approximates to pleural pressure, which changes during the

respiratory cycle. During inspiration, negative intrapleural pressure causes the intrathoracic airway to be splinted open. During expiration, positive intrapleural pressure compresses the intrathoracic airway. Therefore, in intrathoracic airway obstruction (for example in lower tracheal stenosis) typically there is upper airway collapse during expiration and flattened expiratory flow volume curves, but the inspiratory flow volume curve remains normal.

The reverse is true in the fairly compliant extrathoracic airway that is not exposed to intrapleural pressure. Inspiration results in collapse of the extrathoracic upper airway, as the airflow acceleration into the lungs reduces intraluminal pressure. Extrathoracic airways obstruction (upper or mid tracheal obstruction) therefore typically causes airway collapse during inspiration, with flattening of the inspiratory flow volume curve. The force of expiration opens the extrathoracic airway usually resulting in a normal expiratory curve. The expiratory curve may become flattened when there is significant extrathoracic obstruction, resulting in reduced peak airflow rates. Schematics of the classical flow-volume diagrams are displayed in *Figure 1*.

Radiological imaging

Computed tomography (CT) is the radiological modality most often used to image the trachea. Dedicated tracheal protocols allow the acquisition of thin slices through the upper airways. With standard chest protocols, tracheal disease is easily underestimated. "Virtual endoscopy" procedures can be performed using CT images constructed during post-processing, with no additional radiation burden (*Figure 2*). The advantages of virtual endoscopy include the capability to view non-traditional perspectives, to

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Figure 2 Virtual bronchoscopy images of the normal proximal (A) and distal (B) trachea and tracheal stenosis (C).

provide volumetric analyses and to apply automatic feature recognition software (15).

CT is useful for diagnosing tracheal disease, identifying the precise anatomical location, the characteristics of the lesion and the extent of disease, including distal airway patency and local vascular anatomy. When tracheal lesions are visualised in cross section, it is possible to assess whether they are circumferential or incomplete, in a single plane (web like) or in three dimensions, like a cork-screw. CT windows also include the wider chest and may provide supportive evidence of an underlying diagnosis.

The combination of axial imaging, multiplanar reformating, and 3-dimensional rendering is useful prior to tracheal intervention, especially when there is significant anatomical distortion or airway narrowing (16).

Bronchoscopy

Flexible bronchoscopy is often performed in the diagnostic work up for symptoms that are subsequently identified as tracheal in origin. Flexible bronchoscopy is however best avoided due to the risk of precipitating acute, complete airway obstruction or proximal haemorrhage. Rigid bronchoscopy is preferred for evaluating stenotic lesions in the trachea and the advantages over flexible bronchoscopy for diagnosis and therapy which will be discussed further below.

Management

Non-acute airway obstruction

In the non-acute setting, initial management should target ongoing tracheal insults such as inflammation or infection, to retard disease progression. Inflammatory conditions such as collagen vascular disease may respond to steroid or immunomodulatory therapies. Airway infection can be

difficult to control and identification of the pathogenic organism is key. Recurrent pathogen isolation may prompt long term antibiotic prophylaxis as oral or nebulised therapy. Airway clearance is crucial and can be enhanced by the use of mucolytic agents such as carbocysteine, nebulised therapy with saline and/or N Acetyl Cysteine and chest physiotherapy.

Acute airway obstruction

When patients present acutely with significant upper airways obstruction, supportive measures may be necessary and include the commencement of an inspired Helium-oxygen (Heliox) mixture. Heliox is less dense than oxygen and nitrogen. In accordance with Reynold's equation, reducing the density of the inspired gas has the effect of predisposing to laminar flow and this can be used to improve airway dynamics in the short term.

Definitive management

Most significant tracheal stenoses necessitate interventional bronchoscopy or surgical resection. Definitive management should be planned with the input of the multi-disciplinary team. It is the nature of patients with tracheal pathology that their underlying disease or history of intensive care admission may make them high risk surgical candidates; thus, endotracheal intervention is often preferable (17). All patients should, however, be considered for tracheal surgery.

Interventional bronchoscopy does not preclude future surgery in most cases and may optimise potential surgical candidates. Lower surgical success rates are evident if the patient has had previous tracheal surgery, but previous laser therapy does not affect surgical outcome (3).

The most frequent complication of tracheal resection and reconstruction is granulation tissue formation at the

anastomotic site. Since the introduction of novel suture materials in 1978, the complication rate has fallen to 1.6% (18). It is possible to treat granulation tissue at the anastomotic site with endotracheal therapy.

Interventional bronchoscopy

Background

Interventional bronchoscopy should ideally be performed in specialist centres, with the support of experienced, consistent multi-disciplinary teams. Globally, interventional bronchoscopy is most commonly performed using intravenous awake sedation, local anaesthesia and the flexible bronchoscope. Rigid bronchoscopy under general anaesthesia has increased in popularity over the last two decades (19,20) but widespread adoption of the technique is limited by relative operator inexperience and a lack of available training. Rarely, if a tracheal stenosis is high, in close proximity to the vocal cords, a laryngeal mask and flexible bronchoscopy is indicated to visualise and treat the trachea.

Strengths and potential limitations of rigid bronchoscopy

Unlike flexible bronchoscopy, which relies upon the patient's own, potentially unstable airway and ventilation, rigid bronchoscopy offers a controlled, ventilated airway under general anaesthetic, with the support of a cardiothoracic anaesthetist (21). Rigid bronchoscopy is therefore preferable for patients with severe respiratory disease who can be poorly tolerant of flexible bronchoscopy. The use of general anaesthesia also has the benefit of creating an immobile field, free from cough, allowing intervention to be performed more safely (22).

Biopsy or airway intervention during flexible bronchoscopy risks airway haemorrhage with potential compromise of both lungs, rendering the patient hypoxic. Rigid bronchoscopy offers a potentially safer means of obtaining a tissue diagnosis (23). Using the rigid bronchoscope it is possible to apply direct pressure to bleeding lesions, and to apply adrenaline soaked gauze using rigid forceps to tamponade the bleeding source, if direct application of adrenaline solution is not sufficient. Single lung isolation with the rigid bronchoscope can also be used to protect the non-bleeding lung if significant haemorrhage occurs.

The bronchoscope barrel can be used to dissect tissue or dilate tracheal stenoses directly, with excellent access for instrumentation with dilators or stents under direct vision. Rigid bronchoscopy minimises procedure times for endotracheal intervention. The median time to stent deployment is 12 minutes at our institution using rigid bronchoscopy (24).

In a specialist centre with a highly trained and experienced team, low complication rates are seen with rigid bronchoscopy. Potential complications include dental trauma, vocal cord trauma/inflammation and airway haemorrhage. Pneumothorax is a risk due to tracheal instrumentation and positive pressure ventilation but our local experience suggests rates of pneumothorax are less than 1% (based on review of >500 rigid bronchoscopy procedures) (24).

Performing rigid bronchoscopy

The rigid bronchoscope is a hollow, tapered metal tube, with distal side-holes along the body for optimal ventilation. The patient is positioned supine with their neck extended. The pharynx, larynx and trachea are aligned in order to insert the rigid tube, taking care to protect the teeth and vocal cords from trauma.

The lumen of the rigid bronchoscope is used for direct vision. Intervention is performed using rigid instruments passed through the rigid bronchoscope. A flexible bronchoscope is passed through the lumen of the rigid bronchoscope to better visualise segmental airways or to see beyond a narrowed trachea. Some centres use special thin flexible bronchoscopes for this purpose (25). The flexible bronchoscope is also utilised for laser therapy.

Endotracheal intervention

There is an overlap between the techniques used to treat tracheal and bronchial obstruction (26). Options include airway dilatation, tissue destruction and stent insertion, each of which is detailed below.

Airway dilatation

Dilatation is achieved with lubricated bougies of increasing diameter applying radial pressure circumferentially to the narrowed airway. Balloon dilatation is an alternative method. The flexible then rigid bronchoscope can also be used to perform blunt dissection and dilatation of stenosed areas under direct vision.

With all dilating techniques, it is imperative to identify the path of the true airway lumen. It is easy, especially when
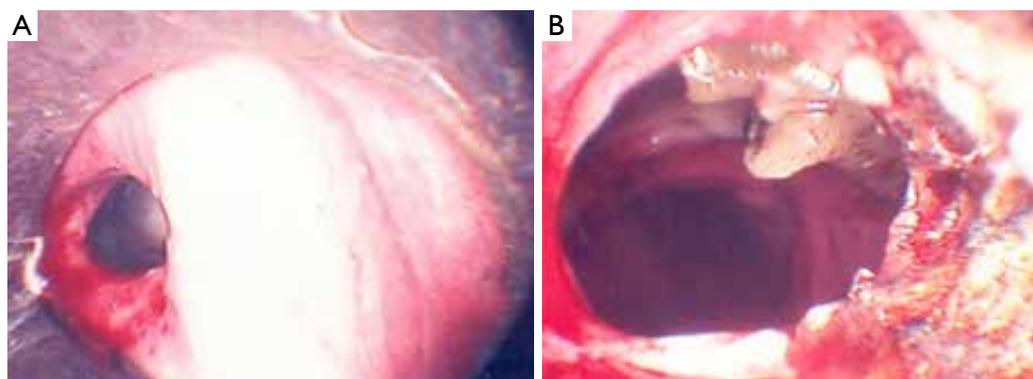
Figure 3 The endoscopic images of tracheal stenosis before (A) and after (B) Nd Yag laser therapy.
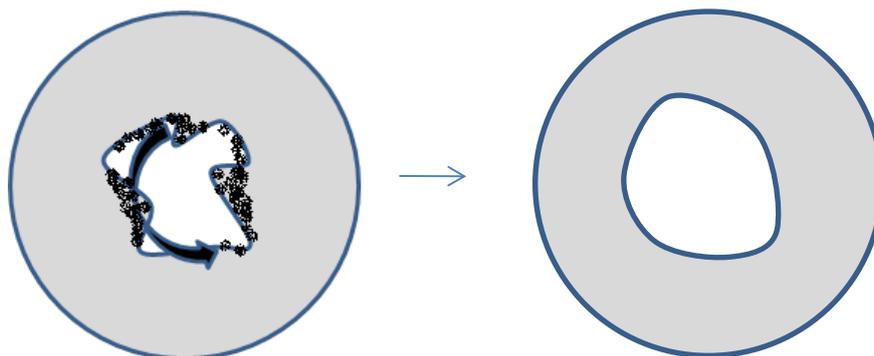
Figure 4 The technique of Nd Yag laser therapy. The laser fibre is moved in a circumferential fashion, starting from the centre of the airway.

the trachea is distorted, to lose sight of the true lumen, risking airway perforation. Pre-operative imaging is useful to define patient anatomy.

Dilatation alone is very rarely a definitive therapy and re-stenosis usually occurs. Dilatation may be used in combination with other therapeutic techniques such as laser ablation and stent insertion, and can be repeated as necessary (NICE guideline IP938).

Tissue destruction

Once the true airway lumen has been identified, it is usually preferable to destroy and physically remove diseased tissue (*Figure 3*). The most rudimentary method of tissue destruction uses forceps to mechanically remove tissue from the trachea. Techniques used to effect tissue destruction include laser therapy, argon plasma, brachytherapy, electrocautery and cryotherapy. Most centres prefer laser therapy, of which the neodymium: yttrium-aluminum-garnet (Nd Yag; Nd: Y3Al5O12) laser is the most commonly used (19,27-29).

Nd-Yag laser energy is delivered via fibres inserted into the working channel of the flexible bronchoscope, using the rigid bronchoscope as a stable airway. The fibres can either be contact or non-contact and are used to devitalise or resect diseased tissue whilst assisting with haemostasis. Nd-Yag laser has a wavelength of 1,064 nm, which is in the invisible photo spectrum. A red light is therefore used to direct application. The bronchoscopist should always apply laser energy parallel to the central airway to avoid unintended trauma to local structures. Energy should be applied in a circumferential motion (*Figure 4*), using 1-5 seconds laser pulses. A circumferential as opposed to radial approach is preferred to open the airway in malignant and benign disease, to ensure good visualisation of the distal airway whilst improving airflow. The lowest possible power is recommended. Our recommended practice is to use a power of 15-20 watts in the trachea, and lower power distally.

Following laser treatment, the airway lumen may still appear narrowed. The effects of the treatment
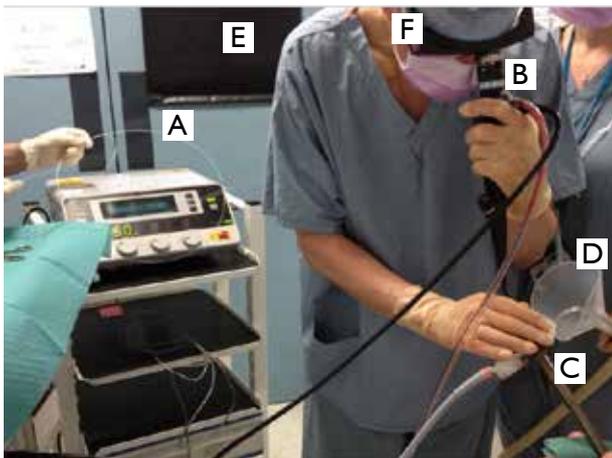
Figure 5 Nd Yag laser therapy. Laser fibre (A) to be inserted through the working channel of the flexible bronchoscope (B), passed through the lumen of the rigid bronchoscope (C). Laser fume extraction device (D), protective curtain (E) and eye wear (F) can be seen.
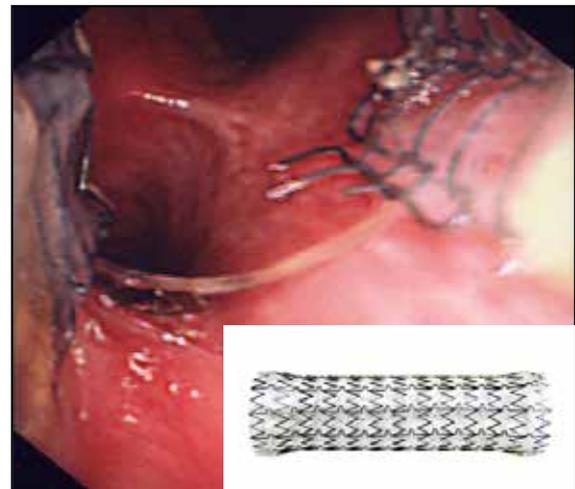
Figure 6 Tracheal metal stent fracture. Endotracheal view of a fractured covered metal tracheal stent and inset picture demonstrating an expanded metal airway stent.

Table 1 Potential complications of endotracheal stent insertion (27,31-39).

Potential complications of endotracheal stent insertion
Mucous plugging
Stent migration
Halitosis
Cough
Obstructing granulation tissue formation
Stent fracture (Figure 6)
Bacterial colonisation/recurrent infection
Fistula formation
Airway malacia (after removal)

continue for days to weeks after the initial application. The bronchoscopist must therefore refrain from being too aggressive with laser therapy. During and after laser treatment, it is important to clear devitalised tissue from the trachea and distal airways. Aspiration is usually sufficient but manual forceps can also be used. The patient will cough up any remaining or further tissue that sloughs away over the coming days.

Personnel should be trained in the use of laser and a committee responsible for laser usage, maintenance and safety should be established and meet regularly. All staff within the potential laser field should wear protective eyewear. The operating room should be adapted for laser therapy with protective curtains, barriers and warning signs at all entry points, and a laser fume extraction device used (*Figure 5*). Inspired oxygen concentration should be less than 40 percent and ventilation should be ceased during laser pulses to reduce the risk of airway fire. If a laryngeal mask is used during laser therapy this should be inflated with saline rather than air, to reduce fire risk.

Reported complications of laser treatment include haemorrhage, airway perforation and airway fire. However, published case series report overall complication rates below 1% in approximately 7,000 treatments (27). With safety measures in place, laser therapy is an excellent and reliable way of treating tracheal stenosis.

There is no limitation to the amount of times laser therapy can be performed. Nearly all patients require more than one endotracheal treatment to achieve long term airway patency. Tissue regrowth can be significantly slowed or halted by serial treatments, with the chance of success increasing after each treatment (30).

Endotracheal stenting

Endotracheal stents are used to provide structural support to the airway and to maintain airway patency. They are, however, foreign bodies in the airway and disrupt mucociliary clearance. The complications of stent placement are listed in *Table 1* and stent fracture is displayed in *Figure 6*.

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Metal and silicone stents are available. Metal stents come with or without a silastic or polyurethane covering which is used to minimise tissue growth when intrinsic tracheal disease is present. The covering is purposefully absent at either end of the stent to allow the stent to anchor to the mucosa and reduce stent migration but re-growth or new tissue growth may occur in these areas.

Historically, metal stents expanded in an unpredictable and uneven manner, resulting in local airway ischaemia, granulation tissue formation, airway perforation and stent migration. The titanium stents in current use are lighter, easier to insert and demonstrate more uniform self-expansion. The application of a more consistent radial force to the airway means perforation, ischaemia and migration are less common and stronger forces can be withstood. There is also a greater availability of stent sizes.

Silicone stents result in a lesser local inflammatory response than metal stents, reducing granulation tissue formation. Silicone stents are therefore easier to remove but have a high risk of stent migration which limits their use (40,41).

When selecting a tracheal stent, it is desirable to use the greatest diameter stent possible. Selection will depend on patient size and disease extent after optimal airway remodelling. In general, airway stents deployed in tracheal disease are between 40-120 mm in length and 14-24 mm wide.

A stent should not be placed when there is active infection as this will promote granulation tissue formation. Treatment of bacterial colonisation in long term airway stents appears useful (42). Our centre routinely offers five days prophylactic oral antibiotics post stent placement.

Stent migration, especially in proximal lesions approaching the vocal cords, can acutely threaten the airway. External fixation of silicone stents has been trialled but with limited success and use, mainly due to cumbersome techniques (43,44). With careful prospective surveillance of metal tracheal stents there is usually minimal risk of migration and no requirement for stent fixation.

Concurrent tracheal and bronchial disease can be managed using Y-shaped silicone stents. The use of Y-shaped stents is limited by recurrent stent obstruction and infection. It is believed the stent structure results in excessive airway friction and mucociliary clearance disruption, with granulation tissue overgrowth and mucus impaction (45). Simultaneous stenting of the trachea and bronchi can be

performed with metal stents, when necessary.

Following stent insertion, surveillance bronchoscopy is indicated in both malignant and benign disease (46). This facilitates early identification and management of complications. Relying on history and examination alone for surveillance is potentially hazardous due to the paucity of symptoms and signs before severe tracheal disease development. Treatment of peri-stent granulation tissue is most commonly addressed by laser therapy (20,31). Timely identification and treatment of airway infection is also crucial.

Indications for stent insertion

In malignant tracheal disease, stenting (with a covered stent) is used to reduce the occurrence of rapid, life threatening disease progression (47). Stenting is also indicated in malignant tracheal fistulae, even if there is no luminal compromise due to tumour bulk. Stents are used to physically obstruct the fistulae, palliating symptoms and protecting the large airway. Stenting for benign tracheal fistulae may be performed in non-operable disease.

Indications for tracheal stent placement in benign disease are less clear than for malignant disease, with varying practices seen worldwide. This is due to the better long term prognosis of individuals with benign pathology, the difficulty removing airway stents, and the reported complications of their use. Endotracheal stent insertion for benign disease should only be considered after airway remodelling by tracheal dilatation and/or tissue destruction has failed to effectively sustain airway patency.

The US Food and Drug Administration recommended in 2005 that metal stents should not be used for benign disease unless absolutely necessary (48). A major concern raised was turning operable cases into inoperable cases (36). Nevertheless, stenting does have a role as a bridge to surgery, enabling optimisation of a patient's functional and physical state prior to surgical intervention.

Tracheal stents for airway malacia should only be considered when patients are symptomatic and airway collapse is greater than 60%. The dynamic radial forces in malacia lead to higher stent complication rates, including metal fracture (49). A further problem with stenting these patients is recognising where to stent, as often long segments are involved. Extensive airway stenting risks higher occlusion rates due to widespread disruption in mucociliary clearance. When a stent is too short for the involved segment this risks displacing airway collapse to the

distal unsupported airway, failing to improve or worsening airway dynamics and symptoms.

Stent removal

Metal stents should be considered permanent as they remodel into the airway by granulation tissue growth and epithelialisation (9,32,50). The longer a stent remains *in situ*, the lower the chance of successful removal. Nevertheless, stent removal has been performed successfully in tracheobronchial disease using both rigid bronchoscopy (51-53) and flexible bronchoscopy (54).

Silicone stents are more easily removed than metal stents and so may lend themselves to short term placement if planned, despite their high migration rates. Research is on-going to produce a fully degradable tracheal stent which can remain *in situ* (55).

Airway management with a tracheal stent *in situ*

Great care should be taken to avoid damaging any tracheal stent if intubation is necessary. It is recommended to use a flexible bronchoscope to ensure that the endotracheal tube is sited above or within the stent lumen (56).

Strong consideration should be given to using rigid bronchoscopy to guide placement of percutaneous tracheostomy in complex tracheal disease (including tracheal stenosis or when a tracheal stent is *in situ*). Percutaneous tracheostomy using rigid bronchoscopy has been previously described (57,58) including where the endotracheal tube is removed and replaced by the rigid bronchoscope (59). The benefits to this approach include better visibility of the complex airway and/or stent, guide wire location, reduced risk of cuff rupture during cannulation and ease of haemostatic control.

Long term tracheostomy

Long term tracheostomy may become necessary for patients with complex tracheal disease. Commonly a Montgomery T tube is placed through a tracheostomy which serves as both a tracheal stent and tracheostomy tube. Tracheostomy is usually reserved for non-surgical candidates, after endotracheal therapy has become complicated and/or requires too frequent procedures, produces suboptimal clinical response or is anatomically too complex to perform safely. Tracheostomy can also be used as a bridge to tracheal surgery or as an adjunct to surgery. Our practice suggests patients are disinclined to tracheostomy, mainly due to negative cosmetic effects, and consider this a last resort.

Interventional outcomes in tracheal obstruction

Malignant disease

There is no randomised controlled trial evidence regarding the use of tracheal intervention in malignant disease due to the ethical challenges in patients requiring life-saving intervention or palliation. The impact of tracheal intervention on survival cannot, therefore, be quoted accurately. Studies have, however, consistently demonstrated that stenting can improve symptoms of breathlessness, quality of life and lung function in malignant disease. Data supports the use of metal covered stents to achieve success rates from 82-97% in these parameters (9,34,40,46,50,60-64). Importantly, improvements in performance status following stenting can open avenues to other therapies for malignancy, potentially improving outcomes further.

Benign disease

Successful short and long term outcomes using a combination of controlled dilatation and/or Nd-Yag laser therapy to destroy endotracheal tissue in tracheal stenosis have been published (28,30,65-68). Despite concerns regarding the use of tracheal stents in benign disease, there are a number of supportive case series and reports in the literature (31,32,35,68,69).

Early studies suggested that endotracheal treatment was less effective for circumferential disease and for stenoses greater than 1 cm in length (70). Recent studies have demonstrated that involvement of the cricoid cartilage and stenoses over 3 cm are associated with a reduced chance of success (71). Time from tracheal stenosis development to first intervention is also important. One study in post intubation tracheal stenosis established that 90% of patients who had intervention within six months of extubation had a positive outcome compared to 61% of those with a longer delay before intervention (72).

Galluccio *et al.* proposed the classification of tracheal stenosis into simple and complex, with simple stenoses defined as those less than 1 cm in length with no associated tracheomalacia or loss of cartilaginous support. Using this classification, silicone stent insertion as part of an endoscopic approach achieved airway patency in 96% of simple lesions at two years follow up but only 69% of complex lesions (30). When considering the removal of short term silicone stents, higher success rates have been seen when sizeable air pockets (longer than 1 cm) between

the stent and tracheal wall are visualised at CT (in post tuberculosis tracheobronchial stenosis) (73).

Although our centre strongly advocates the use of rigid bronchoscopy for endotracheal intervention, other centres have described using flexible bronchoscopy to intubate, dilate and stent patients with tracheal stenosis without complication, in limited patient series (74).

Patient selection for airway stenting in tracheobronchomalacia is crucial as there must be limited disease and a strong enough, supported airway distal to the stent to avoid collapse. As discussed the complication rates of stent insertion in tracheomalacia are higher and when there is malacia from loss of cartilaginous support in tracheal stenosis this reduces the chance of a successful outcome (30,70). However, when patients are carefully selected, studies have demonstrated that patients can achieve relief from breathlessness and an improved quality of life (75,76).

Benign tracheal stenosis has been successfully treated with tracheostomy at long term follow up, including tracheostomy tube placement through tracheal stents remodelled into the airway (36,77).

Summary

Tracheal disease resulting in upper airways obstruction can be life threatening and is an important diagnosis to consider early. A thorough history to identify predisposition to tracheal disease is necessary, with a high clinical index of suspicion directing comprehensive investigation. Prompt treatment of concurrent airway infection is crucial.

Due to the aetiology of tracheal obstruction, the patients are often poor surgical candidates and patients seldom wish to pursue long term tracheostomy. Fortunately, non-resectable tracheal disease can be successfully treated with interventional rigid bronchoscopy to restore airway patency using debulking and/or dilatation techniques. Tracheal stenting is often performed in malignant disease to protect the airway but should be carefully considered in benign disease as stent removal can be difficult. We advocate the use of covered metal stents when stenting is required for tracheal stenosis, due to their infrequent migration ahead of silicone stents. With regular follow up, including surveillance repeat bronchoscopy, endotracheal intervention can achieve long term success for patients with tracheal disease.

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Ultrasound techniques in the evaluation of the mediastinum, part 2: mediastinal lymph node anatomy and diagnostic reach of ultrasound techniques, clinical work up of neoplastic and inflammatory mediastinal lymphadenopathy using ultrasound techniques and how to learn mediastinal endosonography

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Abstract: Ultrasound imaging has gained importance in pulmonary medicine over the last decades including conventional transcutaneous ultrasound (TUS), endoscopic ultrasound (EUS), and endobronchial ultrasound (EBUS). Mediastinal lymph node (MLN) staging affects the management of patients with both operable and inoperable lung cancer (e.g., surgery *vs.* combined chemoradiation therapy). Tissue sampling is often indicated for accurate nodal staging. Recent international lung cancer staging guidelines clearly state that endosonography should be the initial tissue sampling test over surgical staging. Mediastinal nodes can be sampled from the airways [endobronchial ultrasound combined with transbronchial needle aspiration (EBUS-TBNA)] or the esophagus [endoscopic ultrasound fine needle aspiration (EUS-FNA)]. EBUS and EUS have a complementary diagnostic yield and in combination virtually all MLNs can be biopsied. Additionally endosonography has an excellent yield in assessing granulomas in patients suspected of sarcoidosis. The aim of this review in two integrative parts is to discuss the current role and future perspectives of all ultrasound techniques available for the evaluation of mediastinal lymphadenopathy and mediastinal staging of lung cancer. A specific emphasis will be on learning mediastinal endosonography. Part 1 deals with an introduction into ultrasound techniques, MLN anatomy and diagnostic reach of ultrasound techniques and part 2 with the clinical work up of neoplastic and inflammatory mediastinal lymphadenopathy using ultrasound techniques and how to learn mediastinal endosonography.

Keywords: Guidelines; recommendations; lung cancer; sarcoidosis; staging; endoscopic ultrasound fine needle aspiration (EUS-FNA); endobronchial ultrasound combined with transbronchial needle aspiration (EBUS-TBNA); training

Introduction

For a thorough mediastinal nodal evaluation including tissue sampling, a variety of techniques are available: endoscopic techniques (e.g., bronchoscopy), radiological methods (e.g., computed tomography, fluoroscopy, and magnetic resonance imaging), nuclear medicine techniques (e.g., positron emission tomography) and surgical procedures (e.g., mediastinoscopy and video-assisted thoracoscopy). Additionally ultrasound-derived techniques have been introduced that have changed the workflow in the evaluation of mediastinal diseases. Endobronchial ultrasound combined with transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound fine needle aspiration (EUS-FNA) have replaced surgical staging as the initial test of choice for mediastinal tissue evaluation (1-15). Regardless of its numerous advantages, ultrasound-derived techniques are still not utilized to their full potential in respiratory medicine.

The aim of this review in two integrative parts is to discuss the current role and future perspectives of ultrasound techniques for staging of lung cancer and for the evaluation of mediastinal lymphadenopathy. Part 1 deals with an introduction into ultrasound techniques, and part 2 does with the mediastinal lymph node (MLN) anatomy and diagnostic reach of ultrasound techniques, the clinical work up of neoplastic and inflammatory mediastinal lymphadenopathy using ultrasound techniques and how to learn mediastinal endosonography.

MLN anatomy and diagnostic reach of ultrasound techniques

To ensure efficient performance of all mediastinal ultrasound techniques, it is important to have a profound knowledge of mediastinal anatomy and insight how ultrasound images relate to the different nodal stations. According to the International Association for the Study of Lung Cancer (IASLC) classification MLN are divided into different lymph node regions (16). A more anatomically detailed description is given in the following paragraph. The supra-aortic region is defined as the compartment directly above the aortic arch, excluding the area posterior to the trachea, the right paratracheal region as the compartment anterior and lateral to the trachea below the brachiocephalic trunk and above the right bronchus, the aortopulmonary window as the compartment below the aortic arch and above pulmonary trunk, left pulmonary artery, and left main bronchus, the prevascular region as the compartment

anterior to the ascending aorta, vena cava superior, and pulmonary trunk and behind the upper sternum, and the pericardial region as the compartment anterior and lateral to the heart. In the following paragraphs and *Table 1* mediastinal lymph node stations and their evaluability by ultrasound techniques are summarized.

MLN evaluation by (transesophageal) endoscopic ultrasound (EUS)

EUS-guided biopsy allows excellent LN evaluation mainly of the lower mediastinum including the subcarinal region 7, paraesophageal region 8 and pulmonary ligament region 9. EUS also allows access to the left paratracheal region (4L) and partially to the left hilar region (10L). EUS-FNA of region 5 is safe and effective if lymph nodes are considerably enlarged whereas in small lymph nodes FNA might be more difficult or impossible due to interposition of the pulmonary artery/aorta. The para-aortic lymph nodes (station 6) are even more difficult to assess; biopsy (from above the aortic arch) is often difficult avoiding the large mediastinal vessels (17), and the transaortic approach using a 25G-needle may be used only in selected cases (18). The right sided paratracheal and hilar located LN (2R, 4R, 10R) can only be evaluated when grossly enlarged. This can be explained by the anatomy of the esophagus which is located posterior and left-sided to the air-guiding trachea. Therefore, the trachea prevents visualization of the right sided mediastinal regions (19). The examination technique has been recently summarized in textbooks (20-22).

MLN evaluation by endobronchial ultrasound (EBUS)

EBUS-guided biopsy allows excellent LN evaluation of the right (2R, 4R) and left-sided (2L, 4L) paratracheal and the subcarinal regions (7). In addition EBUS provides also easy bilateral access to the hilar region 10 and to the interlobar region 11. Access to intrapulmonary lymph node regions 12-14 is possible only using radial mini-probes (EBUS-R) (23).

In conclusion EBUS and EUS allow complementary evaluation of almost all MLN localizations and combining both methods virtually all mediastinal nodes can be sampled.

MLN evaluation by transcutaneous mediastinal ultrasound (TMUS)

TMUS allows the standardized examination of the

Table 1 Modified lymph node classification according to the international association for the study of lung cancer (IASLC) and evaluable regions using EUS, EBUS and TMUS

No	Region	EUS	EBUS	TMUS
1	Low cervical, supraclavicular and sternal notch nodes regions	(–)	(–)	+++
2	Upper paratracheal region [left (2L), right (2R)]	+	+++	++
3a	Prevascular region	(–)	+	++
3p	Retrotracheal region	+++	+++	(–)
4	Lower paratracheal region [left (4L), right (4R)]			
	4L	++	+++	(+)
	4R	(+)	+++	++
5	Aortopulmonary window	–/+	–	+(+)
6	Para-aortal region	+	(-)	(–)
7	Subcarinal region	+++	+++	+
8	Lower paraesophageal region	+++	(–)	(–)
9	Pulmonary ligament	+++	+	(–)
10	Hilar lymph nodes	(+)	+++	(–)
11	Interlobar lymph nodes	(-)	++	(–)
12	Lobar lymph nodes	(-)	(++)[a]	(–)
13	Segmental lymph nodes	(-)	(++)[a]	(–)
14	Subsegmental lymph nodes	(-)	(++)[a]	(–)

Explanations: +++, ultrasound evaluation is always possible and FNA is easy to perform; ++, Ultrasound evaluation and FNA are often but not always possible; +, ultrasound evaluation and FNA are sometimes possible; –, ultrasound evaluation and FNA are restricted and only possible if LN is grossly enlarged. In the case of lung cancer, ipsilateral pulmonary LNs (hilar, lobar, segmental and subsegmental) are defined as N_1-LNs, ipsilateral mediastinal and subcarinal as N_2-LNs and supraclavicular and scalenus LNs as well as contralateral mediastinal LNs as N_3-LNs. [a], access to LN regions 12-14 is only possible using radial miniprobes (R-EBUS)! EUS, endoscopic ultrasound; EBUS, endobronchial ultrasound; TMUS, transcutaneous mediastinal ultrasound; FNA, fine needle aspiration.

supra-aortic region, prevascular region, right sided upper and lower paratracheal regions (regions 2R, 4R), aortopulmonary window (region 5) and subcarinal region (region 7) under most circumstances (24-30). In addition the precardial region can be easily evaluated.

Clinical work up of mediastinal lymphadenopathy using ultrasound techniques

Enlargement of MLNs is a frequent finding in inflammatory and neoplastic diseases. Conventional chest radiography and thoracic computed tomography are first line diagnostic methods to evaluate suspected mediastinal lymphadenopathy (2,31). In addition, ultrasound methods have gained importance mainly due to their ability to guide biopsy and interventions but also to their detailed spatial resolution. Ultrasound methods allow not only size-related criteria as shown for

computed tomography and magnetic resonance imaging but also evaluation of the lymph node architecture (32,33), lymph node vascularity and perfusion (34-37), resistance index (38), lymph node elasticity (39-41) and changes of perfusion under antiangiogenetic treatment (34).

Several studies have tried to define typical ultrasound criteria for malignant MLNs. One North American and one European EUSstudy found MLNs in 86% and 62% of patients with benign diseases and healthy individuals (42,43). Almost all of these normal lymph nodes have a short diameter below 10 mm and a triangular, crescent or oval shape. Other features like homogeneity, central echogenic structure, and contour differed between individuals and nodes. Contrary to what is often claimed, in both studies number and size of MLNs did not differ between smokers and non-smokers (42,43). Catalano *et al.* (44) in 1994 in a cohort of 100 patients with esophageal cancer
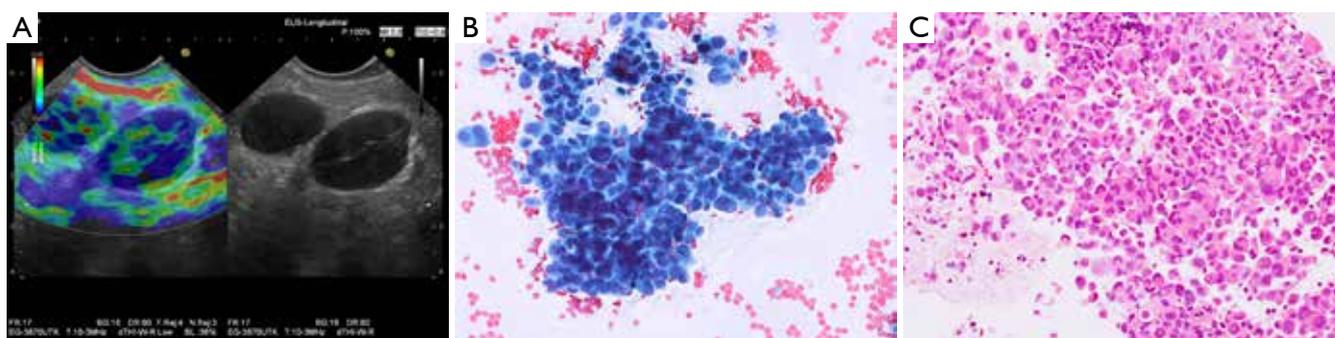
Figure 1 Hypoechoic periesophageal lymph nodes with compression of the hyperechoic hilum in a 49-year-old female patient 6 months after surgery for NSCLC. Elastography shows high and heterogeneous stiffness compared to surrounding connective tissue (A). EUS-FNA was performed using a 22 Gauge-aspiration needle. Smear cytology (B) (Papanicolaou, ×200) and histology (C) (hematoxylin-eosin, ×200) showed poorly differentiated adenocarcinoma. Molecular analysis of the aspirate proved KRAS-mutation in exon 2, but wild type of exon 3 and 4, wild type of exons 2, 3 and 4 of NRAS, wild type of EGFR-exons 18, 19, and 20 and no EML4/ALK-transition. Therefore, a targeted therapy was not possible. Cytological and histologic images: courtesy Dr. K. Zels, Königs Wusterhausen, Germany. EUS-FNA, endoscopic ultrasound fine needle aspiration.
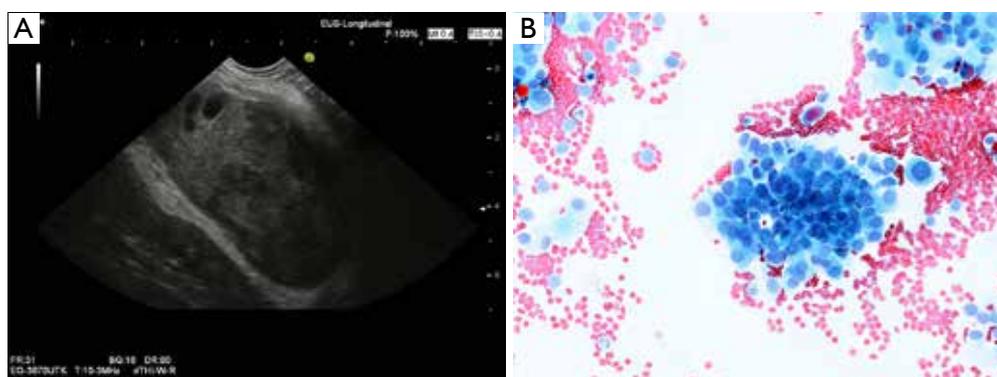
Figure 2 Large hypoechoic, heterogeneous mass lesion of the left adrenal with small cysts in a male patient with radiological suspicion of peripheral lung cancer (A). EUS-FNA was performed using a 22 Gauge-aspiration needle. Smear cytology (B) (Papanicolaou, ×200; immunocytochemistry: CK7 and EMA positive, CD56 and CK5/14 negative) gives proof of poorly differentiated adenocarcinoma, consistent with the clinical suspicion of left adrenal metastasis of NSCLC. Cytological image: courtesy Dr. K. Zels, Königs Wusterhausen, Germany. EUS-FNA, endoscopic ultrasound fine needle aspiration.

defined endosonographic features predictive of lymph node metastasis: hypo- echoic structure, distinct margin, roundness, and a diameter greater than 10 mm. Additionally, as the number of "malignant" echo features rises, the probability of malignancy increased. Malignancy could be predicted with 100% accuracy when all four features were present (44). These endosonographic criteria have been confirmed in further studies using EUS and EBUS. Additional predictive criteria for malignancy of lymph nodes have been added: absence of echogenic hilar structure and of central nodal vessel, echogenic coagulation necrosis, heterogeneous

echo pattern (33,45-49). However, a definitive classification as either malignant or benign by endosonographic criteria is possible only in approximately 25% of MLNs (50). Classification of lymph nodes by EUS criteria alone is less reliable in mediastinal than in lymph nodes of other anatomical locations. Therefore, especially in MLNs EUS-guided fine needle aspiration (FNA) has a significantly higher accuracy than echo features alone (51) (*Figures 1,2*). However, the probability of malignancy is very low, if none of the malignant lymph node criteria is observed (33,52,53).

From a practical point of view it was suggested that

Table 2 Indications for EUS, EBUS and TMUS in pulmonary diseases modified according to Annema *et al.* (8,23,54)

Suspected lung cancer
   Enlarged mediastinal lymph nodes
   FDG-PET-positive mediastinal lymph nodes
   Primary lung tumor adjacent to esophagus (EUS) or the airways (EBUS)
Staging of NSCLC
   Mediastinal staging (regardless of nodal size at CT)
   FDG-PET-positive mediastinal lymph nodes
   Enlarged (short axis >10 mm) FDG-PET-negative mediastinal lymph nodes
   Mediastinal restaging after neoadjuvant treatment (chemotherapy)
   Suspected mediastinal tumor invasion (T4)
   Suspected left adrenal or celiac lymph node metastasis (EUS)
Evaluation of mediastinal masses
   Solitary (multiple) solid mediastinal masses
   Suspected mediastinal metastases of extrathoracic tumors
Mediastinal lymphadenopathy of unknown origin
   Suspected granulomatous disease (sarcoidosis, tuberculosis)
   Suspected lymphoma

EUS, endoscopic ultrasound; EBUS, endobronchial ultrasound; TMUS, transcutaneous mediastinal ultrasound.

patients without any pathological sonographic lymph node criteria should not be biopsied whereas all other lymph nodes should be considered for biopsy (53).

A summary of possible indications for ultrasound techniques in the evaluation of mediastinal and lung diseases is summarized in *Table 2* (8,23,54).

Lung cancer

Lung cancer is one of the most common malignancies and accounts for very high cancer related mortality. In the absence of distant metastases, MLN staging is the most important factor that affects the management and prognosis of patients with lung cancer. Knowledge of locoregional tumor stage is important for planning the best choice of treatment including surgical resection, radiation and chemotherapy (2,31). Mediastinoscopy and thoracoscopy are invasive techniques and, therefore, should be avoided if not necessary.

Lymph node staging

It is obvious that there is an increasing need for minimally invasive techniques including EBUS and EUS techniques with needle aspiration for MLN staging. It has to be taken into account that results of transbronchial biopsy techniques (TBNA) relying on "blind" biopsy are disappointing (55).

EBUS-TBNA has significantly improved the biopsy results (56-62). Promising results have been shown in a multicentric study of 502 patients with a mean lymph node diameter of 16 mm. The reported sensitivity was 94%, specificity 100%, and the positive predictive value 100% (63). Recent meta-analyses have shown a pooled sensitivity of EBUS-TBNA in the range of 88% to 93% (2,64-67). EUS-FNA has a comparable diagnostic yield. Two recent meta-analyses report on a pooled sensitivity of EUS-FNA in nodal staging of NSCLC of 83% and 89%, respectively (2,68).

EUS-FNA and EBUS-TBNA have a complementary diagnostic reach. In combination with both EUS and EBUS almost all important MLNs can be biopsied (59,62,69). Several studies have shown that a combined EUS- and EBUS-approach ("complete endosonographic mediastinal staging") improves lymph node staging versus each of the techniques alone (7-10,23). The sensitivity of both, EUS and EBUS for MLN staging is around 90% (2,23,67,70-73). Meta-analytic data show a substantial increase in sensitivity for mediastinal nodal staging in patients with proven or suspected lung cancer by combining EBUS-TBNA and EUS-FNA or transesophageal FNA using an EBUS-bronchoscope (EUS-B-FNA). Average increment in sensitivity was 21% compared with the esophageal approach alone (pooled data from seven studies) and 13% compared

with EBUS-TBNA alone (pooled data from nine studies) (5). Similar data were reported in another meta-analysis including only studies comparing EBUS-TBNA and EUS-B-FNA with an increase in sensitivity for lung cancer staging of 11% by combining both techniques *vs.* EBUS-TBNA alone (74). The accuracy of the combined approach using EUS-FNA plus EBUS-TBNA proved to be significantly higher than that of PET-CT alone (90.0% *vs.* 73.6%) (12). A randomized controlled study comparing two approaches to combined endosonographic mediastinal staging (EBUS first *vs.* EUS first) found no differences of efficacy and patient's satisfaction in both groups. However, EBUS-TBNA turned out to be the more efficient primary procedure in endoscopic mediastinal staging of potentially operable lung cancer (13). The published studies mainly include cohorts of patients with a relatively high prevalence of mediastinal lymphadenopathy (median 58% for EUS-FNA and EBUS-TBNA; 33% for the combined approach) (2). In studies with a low prevalence of MLN metastases sensitivity of both endosonographic techniques was considerably lower than in studies with a high prevalence (2). Moreover, it has to be taken into account that enlarged lymph nodes and PET-positive findings are the inclusion criteria for most published studies. Meta-analyses uniformly show that sensitivity of EUS-FNA and EBUS-TBNA for detection of metastatic invasion in patients selected on the basis of CT or PET positive results is significantly higher than in patients with negative CT findings or without any selection of CT or PET (66,68). This underlines the importance of biopsies to identify patients who need neoadjuvant treatment strategies.

The combined use of EUS and EBUS can prevent >50% of scheduled surgical staging procedures by providing tissue proof of advanced disease in patients with suspected lung cancer and enlarged or PET positive lymph nodes (75,76).

The results for re-evaluation after neoadjuvant treatment are generally more skeptical (less than 75%). Due to the reported low negative predicative value, negative lymph node findings should be surgically verified (8).

However, both EBUS and EUS have limitations in excluding malignant lymph node involvement. False negative EUS and EBUS findings occur either to sampling errors (lymph node found and biopsied but metastasis missed) or a detection error (lymph node not found).

EUS and EBUS *vs.* mediastinoscopy

Mediastinoscopy, a surgical staging procedure, has been regarded as the gold standard for a long period of time with a sensitivity of 78% for mediastinal nodal staging (72). The additional use of EUS to mediastinoscopy improved locoregional staging (cT4N2-3). The improved results were explained by the complementary diagnostic reach of various nodal stations and the ability of EUS to assess mediastinal tumor invasion (73). The use of mediastinoscopy after a negative endosonography improved the sensitivity of mediastinal nodal staging from 85% to 94% (8). The question which patients staged negative by endosonography should subsequently undergo surgical staging of the mediastinum is a matter of current discussions. It is recommended that in patients with suspicious lymph nodes on either CT or PET, negative endosonography findings should be surgically verified. In contrast there is evidence that patients with centrally located tumors or suspected hilar abnormalities do not benefit from additional surgical staging (23). However, in a very recent study in patients with suspected single level N1 disease, the sensitivity of EBUS for N2 disease was disappointingly low (38%) (77) suggesting a role for mediastinoscopy. However, in this cohort often only EBUS and not the EBUS-EUS combination was used.

It could be shown that in 10-25% of patients with negative CT and in 5-10% of patients with negative PET subsequent endosonographic examinations verify lymph node metastases (78-81).

In conclusion, a complete endosonography evaluation of the mediastinum is at least as good as mediastinoscopy but is associated with fewer complications and futile thoracotomies (8). Therefore, endosonography (and not mediastinoscopy) qualifies as the initial mediastinal tissue staging test (31). Negative endosonography findings however should be verified by surgical staging (5,82).

Proof of diagnosis

In about one third of patients with suspected lung cancer, conventional bronchoscopy fails to prove the diagnosis. In patients with suspected lung carcinoma adjacent to the trachea or bronchi without mucosal (endobronchial) abnormalities, EBUS is superior to CT for guidance of biopsy. The reasons include a better diagnostic yield and a much lower rate of complications including pneumothorax and bleeding in the case of perivascular tumor growth (83,84). In addition, EUS can be used to biopsy centrally located intrapulmonary periesophageal tumors if conventional methods fail (71). In a group of 123 patients with an undiagnosed but suspected malignant lung lesion (paratracheal, parabronchial, paraesophageal) or with a peripheral lung nodule and PET-positive MLNs who had
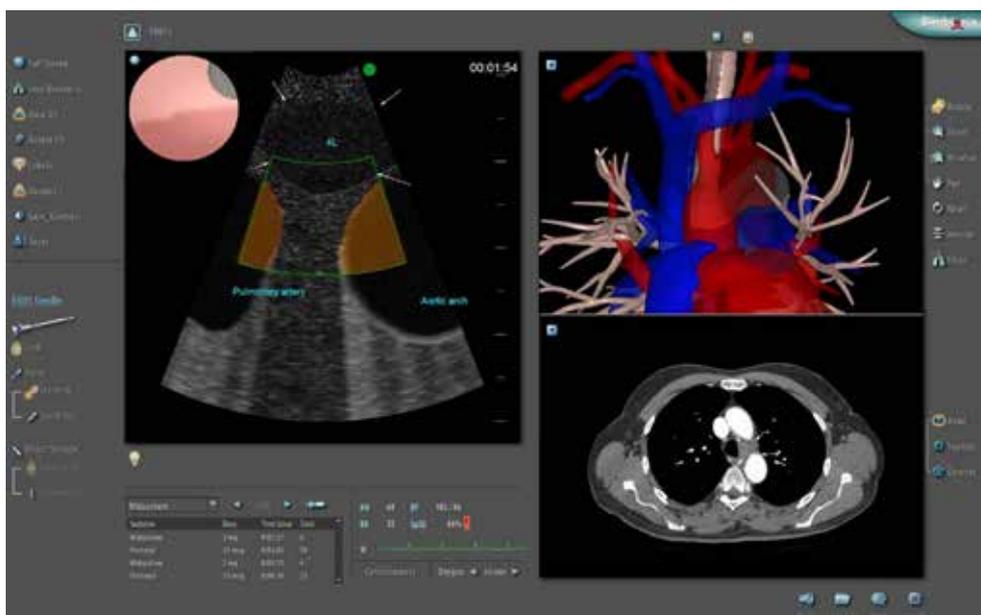
Figure 3 EBUS simulation case (Bronch Mentor-Simbionix). Right lower panel: CT scan of the chest showing a slightly enlarged left paratracheal node (arrows). Left panel: Bronchoscopic screen showing bronchial mucosa with sheath (upper left) and EBUS image showing the station 4L lymph node (arrows) located between the aortic arch and pulmonary artery. Panel right upper corner: anatomical image showing the position of the EBUS scope in the trachea.

undergone at least one diagnostic flexible bronchoscopy or CT-guided transthoracic needle aspiration attempt, EBUS-TBNA and/or EUS-FNA had a high diagnostic efficacy. The endosonographic approach to diagnosis of lung cancer avoided expensive surgical procedures in 106 cases and led to significant cost savings (85).

T-staging

EUS is helpful in selected cases to evaluate T4 (stage IIIB) in the case of possible aortic invasion (70). This specifically applies to invasion in vascular structures. However, more data are needed to make a more definite assessment on this topic.

M-staging

Adrenal gland

In addition, conventional EUS instruments allows the evaluation and biopsy of the left adrenal gland (86-88), which is often involved in metastatic lung carcinoma (89-93). FDG-PET uptake is helpful for detection of adrenal metastases. Focal lesions as the most important imaging sign but also increased size and loss of the typical "seagull shape" are predictors for malignant involvement (89). In addition, the left adrenal gland can be reached and sampled

by EUS-FNA demonstrating a high yield (91,94,95). The right adrenal gland can be assessed using a transduodenal or transgastric approach, which is technically more demanding and sometimes dangerous if a decubitus or right sided position for visualization is required. EUS is inferior to transcutaneous ultrasound (TUS) in the evaluation of the right adrenal gland (96,97). TUS-guided biopsy is recommended (88,98). However, in cases transduodenal biopsy of suspected right sided adrenal metastases have shown to be feasible (99,100). A preoperative bilateral EUS examination and EUS-FNA of the adrenal glands in patients with potentially resectable lung cancer has a high diagnostic accuracy in detecting adrenal metastases (89,101) (*Figure 3*). Recently a few reports in abstract form have reported the use of the EBUS scope for the assessment of the left adrenal gland. However, more data are needed for a more definitive assessment.

Liver

In rare cases liver metastases are detectable only by EUS with CT-negative findings. In such cases the EUS-guided biopsy of the liver is helpful to proof the metastatic spread (98,102,103).

Other infradiaphragmal manifestations

In even more rare cases pancreatic metastases (or infiltration
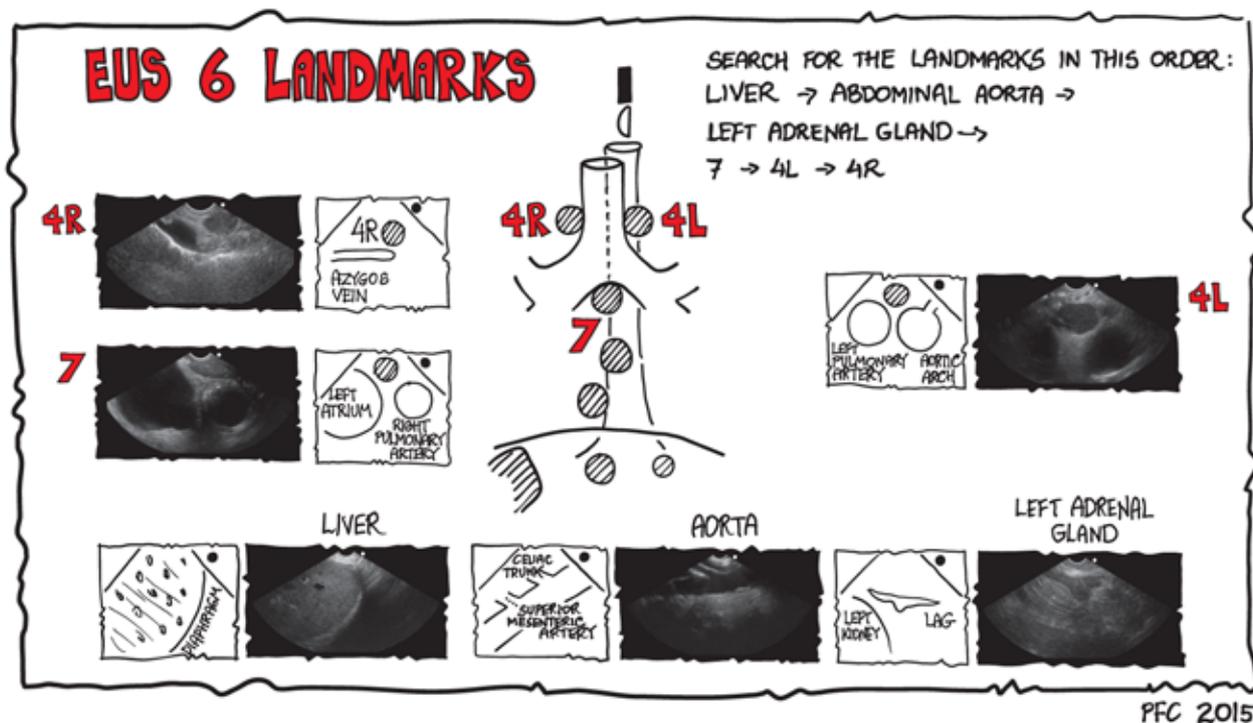
Figure 4 The six landmarks of endoscopic ultrasound (EUS) for lung cancer staging. Paul Clementsen is owner of the copyright.

of celiac or peripancreatic lymph nodes) are detectable only by EUS with CT-negative findings. In such cases the EUS-guided biopsy of the pancreas is helpful to prove the metastatic spread (98,104-107).

Mediastinal staging of extrathoracic malignancies

As has been shown for pancreatobiliary cancer, in up to 10% of extrathoracic malignancies metastasize to MLNs (108). Both EUS and EBUS have been successfully used for the assessment of tumor spread to MLNs (M1 disease) in patient cohorts with various extra-thoracic malignant diseases (109-116). In particular, the usefulness of EUS-guided sampling of MLNs has been reported in the staging of patients with gastric cancer, pancreatic cancer (108,117) breast cancer (118), upper GI cancer (119,120); head and neck cancer (121), colorectal cancer (122), and lymphoma (57,123-129). A recent meta-analysis (five studies, n=533 patients) showed a high value for EBUS-TBNA for the diagnosis of mediastinal and hilar lymph node metastases from extrathoracic malignancy. Pooled sensitivity was estimated 85% with a specificity of 99% (130). Procurement of specimens which are eligible for immunohistochemistry

is important for reliable differentiation between mediastinal nodal metastases of extrathoracic cancer *vs.* non-small cell lung cancer.

How to learn pneumological endosonography

A systematic training in mediastinal endosonography should ideally be based on (I) theoretical knowledge, (II) performance on simulators and (III) supervised performance on patients. Each step should be completed by passing a validated exam before proceeding to the next step. However, there are no commercially available virtual reality simulators for mediastinal EUS-FNA, but it is possible to practice EBUS-TBNA on both the GI Bronch Mentor™ (Simbionix) (*Figure 4*) and the AccuTouch Flexible Bronchoscopy Simulator™ (GE Healthcare). A standardized test including pass/fail-standards has been developed for the GI Bronch Mentor (131,132).

Firstly the trainee should learn to recognize anatomic landmarks and mediastinal vessels (133) by observing the procedure (134,135). The next step is to learn to insert the endoscope and to "produce" the pictures, which is much more difficult than watching an experienced examiner doing
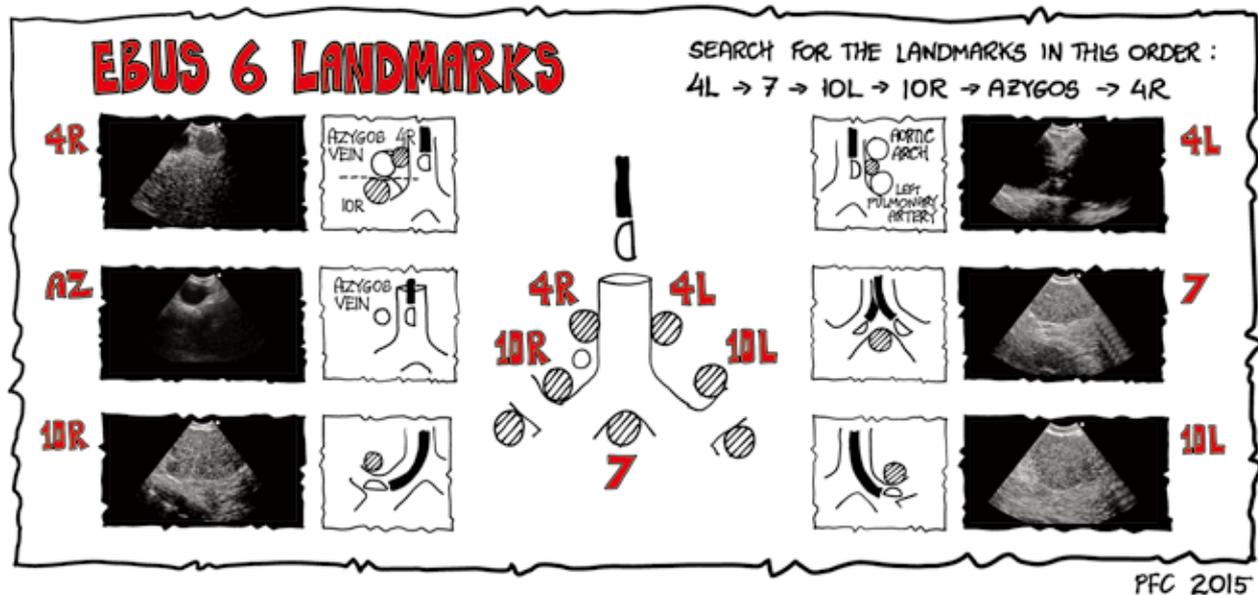
Figure 5 The six landmarks of endobronchial ultrasound (EBUS). Paul Clementsen is owner of the copyright.
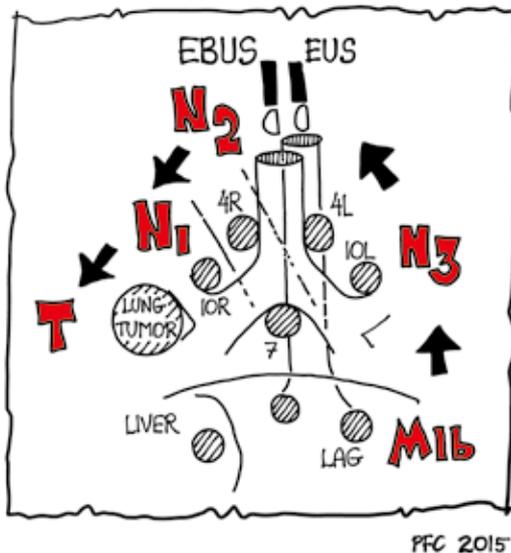
Figure 6 Systematic approach to endosonographic lung cancer staging. Paul Clementsen is owner of the copyright.

the procedure. The final task relates to correct positioning of the transducer, proper use of the needle sheath and handling of the needle when taking fine needle aspirates (123-126,136-140).

After passing a simulation-based test the trainee should perform the initial endosonography procedures in patients under supervision. The learning curve should be monitored by specific tools for assessment, since the number of procedures to obtain competence varies from trainee to trainee (127-129,131,141,142). For EBUS-TBNA, in a multicenter cohort of fellows in pulmonary medicine, the majority of trainees achieved first independent successful performance of EBUS-TBNA following a training protocol that included theoretical education and simulation sessions at an average of only 13 procedures (143). Another study with nine interventional pneumologists failed to observe such a steep learning curve and observed ongoing improvements for lymph node identification by EBUS and EBUS-TBNA skills even after 200 clinical cases (144).

The classical approach is to start by learning the six basic landmarks for EBUS and EUS and to practice finding them in the order mentioned (*Figures 4-6*) (145).

The six EUS landmarks (Figure 4)

- Liver (landmark I): introduce the endoscope into the esophagus and slide down below the diaphragm. Turn the endoscope counterclockwise to find the left liver lobe.
- Aorta (landmark II): turn the endoscope clockwise and find the aorta with the celiac trunk and the superior mesenteric artery.
- The left adrenal gland (landmark III): turn the endoscope
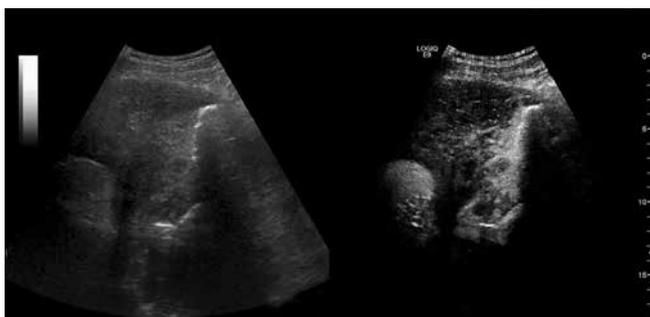
Figure 7 Mediastinal ultrasound using contrast enhanced ultrasound to demonstrate vital Hodgkin's disease by contrast enhancement.

further clockwise, move the transducer a little upwards to find the left adrenal gland (it resembles a small bird, seagull) close to the upper pole of the left kidney.

- Station 7 (landmark IV): retract the endoscope to the mediastinum and find station 7 below the carina close to the left atrium and the right pulmonary artery.
- Station 4L (landmark V): retract the endoscope a few centimeters, turn counterclockwise and find station 4L between the aortic arch and the left pulmonary artery (the vessels resemble the ears of Mickey Mouse).
- Station 4R (landmark VI): turn clockwise until you pass the trachea and find the azygos vein. Retract the endoscope slowly until the vein disappears into the superior cava vein and search for station 4R. If it is of normal size, it will, often hide behind the trachea.

The six EBUS landmarks (Figure 5)

- Station 4L (landmark I): turn the endoscope counter clockwise and find station 4L between the arch of the aorta and the left pulmonary artery.
- Station 7 (landmark II) is found below the carina with the endoscope in the right or the left main bronchus facing medially.
- Station 10L (landmark III) is found looking upwards with the transducer in the left upper lobe bronchus.
- Station 10R (landmark IV) is found looking upwards with the transducer in the right upper lobe bronchus.
- The azygos vein (landmark V): retract the endoscope and find the azygos vein paratracheal to the right.
- Station 4R (landmark VI) is found above the azygos vein. The inferior border of the azygos vein marks the border between station 4R and 10R.

Handling of the endoscope: a few tips and tricks

- Note carefully on the ultrasonic picture, if the endoscope is coming from the right or the left side. "The dot" shows where the proximal part of the endoscope on the ultrasonic picture is located. Avoid confusion with a mirror image.
- With the EUS endoscope, a rotation to the right (clockwise) moves the transducer to the right side of the patient, when the transducer is directed forward, i.e., above the diaphragm. The same rotation will move the transducer to the left side of the patient when the transducer is directed backwards below the diaphragm.
- When performing EBUS, it must be remembered that the view is typically in an oblique direction of 30 degrees, so it can be difficult to get access to the trachea.
- All regions should be inspected systematically with a 360 degrees rotation for every four centimeters. Do not overlook any structures that are not necessarily located according to the two times six landmarks.

Practical advice: systematic approach to endosonographic lung cancer staging

The order of recommended examinations (EBUS, EUS) depends mainly on the side and localization of the tumor determined by the CT findings. Biopsies should be performed under the premise that distant metastases (M1) are excluded first, followed by lymph node staging in the order N3 (contralateral lymph nodes) → N2 (ipsilateral mediastinal and subcarinal lymph nodes) → N1 (ipsilateral hilar lymph nodes) (*Figure 6*). For patients with suspected N2 disease infiltration of only single N2 lymph nodes (N2a, stage IIIA3) has to be differentiated from infiltration of more than one N2 lymph node region, clusters of involved lymph nodes in one or more N2 stations, or large N2 lymph nodes with extracapsular invasion (N2b, Stage IIIA4) (1-3,31,72,146).

Lymphoma

Mediastinal ultrasound

In a retrospective study [40 consecutive patients with Hodgkin's (n=29) and non-Hodgkin's (n=11)] MTUS was clearly superior to chest radiographs and comparable to CT for monitoring patients with mediastinal lymphomas (147) (*Figure 7*). Thymic enlargement due to involvement by Hodgkin disease is more frequently observed than previously reported.
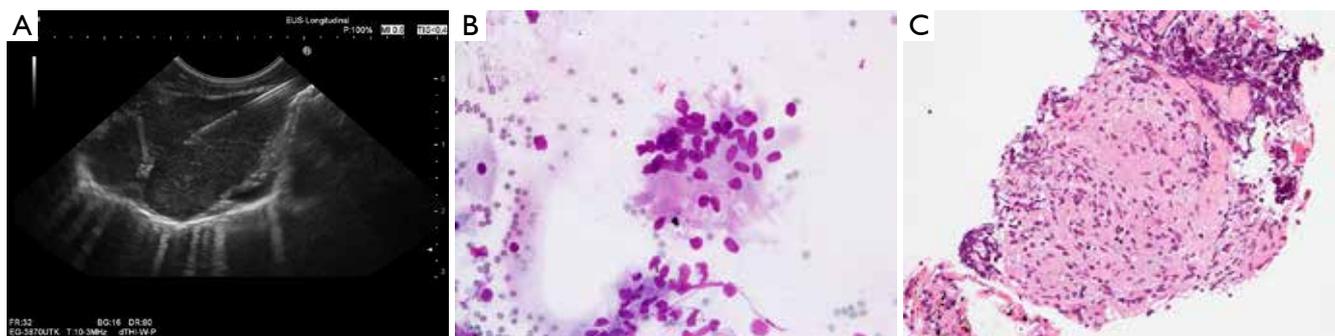
Figure 8 EUS-FNA using a 22 Gauge-aspiration needle of large hypo-echoic mediastinal lymph nodes (station 7) in 50-year-old women with dry cough (A). Smear cytology (May-Gruenwald-Giemsa, ×200) shows groups of epithelioid cells (B), histology (hematoxylin-eosin, ×200) demonstrates typical epithelioid granuloma without necrosis and with some multinuclear giant cells (C). Clinical diagnosis was sarcoidosis. Cytological and histologic images: courtesy Dr. S. Wagner, Königs Wusterhausen, Germany. EUS-FNA, endoscopic ultrasound fine needle aspiration.

Thymic gland involvement is sonographically visible due the hypoechoic structure. In contrast MTUS was not helpful to differentiate the normal-sized typical tongue-shaped thymus from surrounding fatty tissue after treatment due to the same echogenicity of the gland and the surrounding fat (148). Elastography and contrast enhanced techniques might overcome this problem but data are lacking (32).

It is of importance that lymphoma and other tumors in the anterior mediastinum can also be biopsied under ultrasound guidance via a suprasternal and strict parasternal approach. Using the parasternal approach non-visible lymphoma might get visible due to the shifting of the mediastinum from the decubitus to a strict left or right lateral position (22,26).

EUS-FNA and EBUS-TBNA

EUS-FNA and EBUS-FNA have a variable diagnostic yield for diagnosing and subtyping of non-Hodgkin Lymphoma of the posterior and inferior mediastinum. There are good data that EBUS/EUS is useful for the assessment of recurrent lymphoma, for the primary lymphoma diagnosis often a histology specimen—obtained by mediastinoscopy—is needed. However, cell block processing of material obtained by EUS-FNA or EBUS-TBNA may have nearly similar diagnostic yield as histology (149-152). Two large cohort studies demonstrated, that accuracy of EBUS-FBNA for diagnosis of mediastinal malignant lymphoma was 84% and 91%, with correct subtyping possible in >2/3 of cases (150,153).

Inflammatory diseases

Sarcoidosis and tuberculosis
Depending on the geographic distribution, sarcoidosis and tuberculosis are the two most important inflammatory causes of mediastinal lymphadenopathy (109,154-158).

Sarcoidosis
The typical imaging finding of sarcoidosis lymphadenopathy are symmetrically distributed clusters of MLNs around large vessels. The typically oval-shaped lymph nodes may reach a size of up to 60 mm with mixed echogenicity depending on the stage of the disease (156). Color Doppler imaging, contrast enhanced ultrasound techniques and elastography have shown that the lymph node architecture is typically not destroyed and a hilum can be displayed (159-161).

Both EUS-FNA and EBUS-TBNA are suitable for final diagnosis of sarcoidosis (*Figure 8*) whereas pure transbronchial biopsy fails in about one third of cases. Published data indicate that the sensitivity (80-90%) and accuracy of EUS-FNA and EBUS-TBNA are superior compared to simple mucosa biopsies without and with "blind" transbronchial puncture (155,162-165). Special techniques (cytology and cell-block analysis) might even improve the diagnostic yield of ultrasound-guided biopsies (162). A meta-analysis (14 studies including 2,097 patients) showed a diagnostic yield of 79% for diagnosis of sarcoidosis by EBUS-TBNA. Pooled sensitivity and specificity were 84% and 100%, respectively (166).

Complications may be encountered. Mediastinitis with abscess formation has been observed after transesophageal biopsy of MLNs (167). Therefore, prophylactically administered antibiotics may be considered for EUS-guided biopsies but studies on this topic are lacking. Similar complications haven not been observed in EBUS-TBNA, therefore, no prophylactically applied antibiotics are recommended.

In conclusion, for the diagnosis of sarcoidosis, endosonographic techniques are superior to the combination of endobronchial mucosa and transbronchial lymph node biopsies. Besides conventional cytological smears, cell blocks are recommended to increase the diagnostic yield.

Differential diagnoses

Under specific circumstances also depending on geographic and other epidemiological criteria tuberculosis and atypical mycobacteriosis have to be excluded in the case of unclear mediastinal lymphadenopathy. Several studies have shown acceptable diagnostic accuracy for the diagnosis of MLN tuberculosis by EUS-FNA and EBUS-TBNA. Cytopathological criteria, the search for acid-fast bacilli (stained red) using Ziehl-Neelsen-technique or Acridin-Orange-staining as well as culture techniques and PCR are helpful for final diagnosis (157,158,168-170). Concurrent systemic symptoms may be encountered (171).

Besides tuberculosis, atypical mycobacteriosis, sarcoidosis and other granulomatous diseases paraneoplastic "sarcoid like reaction" (SLR) have to be included in the differential diagnosis of granulomatous lymphadenopathy. SLR has been observed in the neighborhood of malignancies as well as sequelae of chemotherapy and radiation. Positron emission tomography (PET) may show false positive results in patients with SLR (172-174).

Mediastinal ultrasound in patients with cystic fibrosis

The respiratory tract is involved in almost all patients with cystic fibrosis and respiratory failure accounts for about 90% of morbidity and mortality in patients with cystic fibrosis. Extrapulmonary manifestations are also often encountered (175). Evaluation of TMUS in healthy subjects and patients with cystic fibrosis demonstrated that the lymph node detection rate in the paratracheal region and aortopulmonary window was significantly higher in patients with cystic fibrosis and the total lymph node volume was larger, respectively. Therefore, mediastinal ultrasound was helpful for the detection of inflammatory activity in patients with cystic fibrosis (176). Similar studies using EUS and EBUS have not been published.

Mediastinal ultrasound in patients with chronic virus hepatitis C

Mediastinal lymphadenopathy can be considered as an extrahepatic manifestation of chronic hepatitis C. TMUS was also able to detect slightly enlarged MLNs in patients with chronic virus hepatitis C. In patients with chronic hepatitis C a trend could be observed, that patients with larger perihepatic lymph nodes also reveal larger MLNs indicating a systemic pathomechanism. The mechanism of lymphadenopathy in the liver hilum (177-179) and mediastinum in patients with chronic hepatitis C and other viral and autoimmune liver diseases is yet unknown (180). Similar studies using EUS and EBUS have not been published. Therefore, normal lymph nodes were detectable more frequently in the paratracheal region and aortopulmonary window of cadavers compared to the respective mediastinal regions of healthy volunteers. A possible explanation of this finding lies in the better image resolution obtained by application of the transducer to the region of interest in cadavers. The difference in age may also have an impact.

Conclusions

Endobronchial, endoesophageal and TUS are complimentary approaches for the evaluation of the mediastinum, in particular in patients with non-small cell lung cancer and with mediastinal lymphadenopathy. All three techniques facilitate tissue acquisition from MLNs or masses for primary diagnosis or staging. Due to their high accuracy and low risk, ultrasound-guided sampling procedures should be considered to substitute for more invasive surgical techniques. Learning ultrasonographic evaluation of the mediastinum should be performed in a systematic manner based on the classical anatomical landmarks.

Acknowledgements

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Ultrasound techniques in the evaluation of the mediastinum, part I: endoscopic ultrasound (EUS), endobronchial ultrasound (EBUS) and transcutaneous mediastinal ultrasound (TMUS), introduction into ultrasound techniques

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Abstract: Ultrasound imaging has gained importance in pulmonary medicine over the last decades including conventional transcutaneous ultrasound (TUS), endoscopic ultrasound (EUS), and endobronchial ultrasound (EBUS). Mediastinal lymph node staging affects the management of patients with both operable and inoperable lung cancer (e.g., surgery *vs.* combined chemoradiation therapy). Tissue sampling is often indicated for accurate nodal staging. Recent international lung cancer staging guidelines clearly state that endosonography (EUS and EBUS) should be the initial tissue sampling test over surgical staging. Mediastinal nodes can be sampled from the airways [EBUS combined with transbronchial needle aspiration (EBUS-TBNA)] or the esophagus [EUS fine needle aspiration (EUS-FNA)]. EBUS and EUS have a complementary diagnostic yield and in combination virtually all mediastinal lymph nodes can be biopsied. Additionally endosonography has an excellent yield in assessing granulomas in patients suspected of sarcoidosis. The aim of this review, in two integrative parts, is to discuss the current role and future perspectives of all ultrasound techniques available for the evaluation of mediastinal lymphadenopathy and mediastinal staging of lung cancer. A specific emphasis will be on learning mediastinal endosonography. Part I is dealing with an introduction into ultrasound techniques, mediastinal lymph node anatomy and diagnostic reach of ultrasound techniques and part II with the clinical work up of neoplastic and inflammatory mediastinal lymphadenopathy using ultrasound techniques and how to learn mediastinal endosonography.

Keywords: Guidelines; lung cancer; sarcoidosis; staging; endoscopic ultrasound fine needle aspiration (EUS-FNA); endobronchial ultrasound combined with transbronchial needle aspiration (EBUS-TBNA); training; endoscopic ultrasound with bronchoscope fine needle aspiration (EUS-B-FNA); transcutaneous ultrasound (TUS)

Introduction

Tissue acquisition of mediastinal lymph nodes is often essential for diagnostic purposes and in case of malignancy, for accurate staging. Malignant mediastinal lymph node infiltration has a major impact on lung cancer treatment, as those patients without malignant nodal involvement are commonly treated with immediate surgical resection of the tumor containing lobe or received radiotherapy with curative intent whereas those with nodal involvement are treated with chemoradiation (1-6). Chest imaging by computed tomography (CT) including intravenous contrast enhancement provides detailed anatomical information of the mediastinum, hilum, and lung parenchyma and chest wall. Fluorodeoxyglucose-positron emission tomography (FDG-PET) scanning, preferable in combination with CT, can provide important physiological information regarding mediastinal nodes and lesions. Due to limitations of the imaging techniques, enlarged or FDG avid nodes should be sampled to prevent over and under staging.

For a thorough mediastinal nodal evaluation including tissue sampling, a variety of techniques are available: endoscopic techniques (e.g., bronchoscopy), radiological methods [e.g., CT, fluoroscopy, and magnetic resonance imaging (MRI)], nuclear medicine techniques (e.g., PET) and surgical procedures (e.g., mediastinoscopy and video-assisted thoracoscopy). Additionally ultrasound-derived techniques have been introduced that have changed the workflow in the evaluation of mediastinal diseases. Ultrasound imaging has gained importance including conventional transcutaneous ultrasound (TUS) of the chest wall, and of pleural effusions (7-9). Nowadays, thoracentesis and chest tube placement is preferably performed following prior sonographic evaluation of the chest.

Ultrasound has been established in the head and neck regions to evaluate cervical and supraclavicular lymphadenopathy (10,11). In addition, transcutaneous mediastinal ultrasound (TMUS) is also able to detect normal and pathological lymph nodes in the deeper located mediastinal region but this knowledge is not widespread and requires special skills. Endobronchial ultrasound combined with transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound fine needle aspiration (EUS-FNA) have replaced surgical staging as the initial test of choice for mediastinal tissue evaluation (4-6,12-23). Regardless of its numerous advantages, ultrasound-derived techniques are still not utilized to their full potential in respiratory medicine.

The aim of this review in two integrative parts is to discuss the current role and future perspectives of ultrasound techniques for staging of lung cancer and for the evaluation of mediastinal lymphadenopathy. Part I is dealing with an introduction into ultrasound techniques and part II with the mediastinal lymph node anatomy and diagnostic reach of ultrasound techniques, the clinical work up of neoplastic and inflammatory mediastinal lymphadenopathy using ultrasound techniques and how to learn mediastinal endosonography.

Introduction into ultrasound techniques

Non-invasive benchmark: CT, PET-CT, MRI

CT is the anatomical standard for the description of intrapulmonary lesions and mediastinal abnormalities. In the evaluation of mediastinal lymph nodes, the clinical significance of CT is less convincing since CT mainly relies on size parameters. Cut off values for the short-axis diameter of 10-15 mm were suggested to define abnormal lymph nodes for decades (24,25) with false positive and false negative findings in about 25% of cases indicating a low accuracy (26-28). In two systematic analyses the cumulative sensitivity of CT in mediastinal staging of non-small cell lung cancer (NSCLC) was estimated to be 55% or 61%, respectively, with a specificity of 81% or 79%, respectively (5,29). The lower the cut-off value the higher the sensitivity can be shown at costs of the specificity (30). The problem of metastases in normal sized lymph nodes seen on the CT scan has already been addressed in some earlier studies (31-36). A morphometric study of 2,891 hilar and mediastinal lymph nodes from 256 patients with NSCLC showed a significant difference of diameter between metastatic and non-metastatic lymph nodes. However, 44% of metastatic lymph nodes were <10 mm in diameter, and of 139 patients with no metastatic lymph node involvement as much as 77% had at least one lymph node that was >10 mm in diameter (36). More than one of four patients with NSCLC had metastasis in the second largest but not in the largest mediastinal node (37). Preliminary results of quantitative CT analysis of shape and texture of mediastinal lymph nodes are promising, showing higher sensitivity for the detection of malignant lymph nodes then sole size measurement (38). In short, lymph nodes with a short axis over 10 mm are considered enlarged, but this does not imply malignant involvement.

Results of PET and of integrated PET-CT have improved

the accuracy of CT for detecting mediastinal lymph node metastases of NSCLC to some degree. In a recent meta-analysis, the pooled weighted sensitivity and specificity of PET-CT in a patient-based group were estimated 76% and 88%, respectively (39). In a prospective multicenter study the increment of accuracy in detecting lymph node metastasis provided by adding PET to CT was approximately 11% on a per-patient basis (40). Furthermore, integrated PET-CT adds value to staging of lung cancer in the evaluation of chest wall invasion, of mediastinal infiltration, and in the detection of occult distant metastases. However, despite combining functional and morphological imaging in one method, PET-CT is not able to solve the problem of nodal size. Results are disappointing, since false positive findings are relatively frequent in large lymph nodes. In one study the sensitivity of PET-CT was significantly higher among enlarged (>10 mm) than non-enlarged (≤10 mm) lymph nodes (74% vs. 40%). On the other hand, specificity (81% vs. 98%) and accuracy (78% vs. 90%) were significantly lower in enlarged compared to non-enlarged lymph nodes (41). Another study from the same group showed that in NSCLC patients who are clinically staged as N2/N3 negative by integrated PET-CT, 16% will have occult N2 disease following resection. The highest rate of occult (PET-CT negative) N2 involvement was found in the infracarinal (64%) and in the lower paratracheal lymph node stations (28%). As independent predictors of occult N2 disease were identified: centrally located tumors, right upper lobe tumors and [18]FDG-uptake in N1 nodes (42). The risk of false-positive PET-findings in hilar and mediastinal lymph nodes is significantly higher in larger lymph nodes and in lymph nodes with a high volume of macrophages and lymphocytes (43). Moreover, there is a correlation of lymphoid follicular hyperplasia with false-positivity of mediastinal lymph nodes in PET-CT (44), illustrating the risk of misjudging enlarged inflammatory and reactive lymph nodes for lymph node metastases by PET-CT. One recent study found concurrent lung disease or diabetes mellitus, histology other than adenocarcinoma, and a high [18]FDG uptake of the primary tumor to be risk factors of false negative results. On the other hand, age >65 years, good differentiation of the tumor and a low [18]FDGE-uptake of the primary tumor were significantly correlated with false positive results (45).

Therefore, lymph node staging using PET-CT is far from equal to pathological staging. In selected patients with negative PET-CT-results for N2/N3 disease as well as in patients with PET-positive mediastinal lymph nodes, lymph node biopsy is still required for final diagnosis before

thoracotomy. In addition to nodal staging, FDG-PET scanning results in the identification of unexpected distant metastasis in up to 5-10% of patients.

Furthermore, it should be mentioned that reimbursement of PET and PET-CT has not been introduced into many health care systems except under a few defined clinical situations (46,47).

The value of MRI in mediastinal imaging is much less compared to the brain, musculoskeletal system, abdomen and pelvis. However, a recent meta-analysis suggested that the accuracy of diffusion-weighted MRI for mediastinal and hilar nodal staging of NSCLC may be comparable to PET-CT (48).

To compare measurements of mediastinal lymph node sizes obtained by CT with those obtained by ultrasound techniques is difficult, because lymph nodes are situated longitudinally in the mediastinum, whereas CT-images are transversally oriented. In contrast, ultrasound allows measurement of lymph node sizes in any plane. Therefore, the sonographically estimated lymph node size correlates closer to the morphometric assessment than to measurements obtained by axial CT (34). Two recent comparative cohort studies found only a weak agreement between thoracic CT and EBUS for size estimation of mediastinal and hilar lymph nodes (49,50). Using EBUS-TBNA, malignant cells were obtained from 24% of lymph nodes initially interpreted as normal in size (50).

Invasive benchmarks: mediastinoscopy and video-assisted thoracoscopy

Minimal-invasive surgical methods for mediastinal staging of NSCLC and sampling of mediastinal lymph nodes are standard cervical mediastinoscopy, video-assisted mediastinoscopy (VAM) and lymphadenectomy (VAMLA), and video-assisted thoracoscopic surgery (VATS). Access to mediastinal lymph node stations, invasiveness and diagnostic yield differ between the particular surgical methods (*Table 1*). VAM allows better visualization and has a better lymph node yield (including the opportunity of performing lymph node dissection) than standard mediastinoscopy (5,51). The major limitation of cervical mediastinoscopy is its inability to access lymph node stations 5 and 6. Therefore, several methods are used to supplement cervical mediastinoscopy as the traditional anterior (parasternal) mediastinotomy (Chamberlain procedure), extended cervical mediastinoscopy (ECM) or transcervical extended mediastinoscopy (TEMLA). VATS is generally limited to

Table 1 Yield and safety of surgical methods to access mediastinal and hilar lymph node stations [data from: (5,51-53)]

Diagnostic yield and safety	Standard mediastinoscopy	VAM/VAMLA	VATS
Accessible LN stations	1, 2 R/L, 3, 4 R/L, 7 (anterior)	1, 2 R/L, 3, 4 R/L, 7	Right-sided VATS: 3 R, 4 R, 7, 8-10 R; left-sided VATS: 5-7, 8-10 L
No access to mediastinal LN stations	5, 6, 7 (posterior), 8, 9	5, 6, 8, 9	All contralateral stations: right-sided VATS: +5, 6; left-sided VATS: +3, 4 L
Diagnostic sensitivity in lung cancer staging	78% (26 studies, 9,267 patients)	VAM: 89% (7 studies, 995 patients); VAMLA: 94% (2 studies, 386 patients)	99% (4 studies, 246 patients)
Morbidity	0-5.3%	0.8-2.9%	0-9%
Mortality	0-0.08%	0%	0%

VAM, video-assisted mediastinoscopy; VAMLA, video-assisted mediastinoscopy lymphadenectomy; VATS, video-assisted thoracoscopic surgery; LN, lymph node.

the evaluation of one side of the mediastinum. It has a major role in diagnosis and treatment of benign and malignant pleural disease as well as of solitary pulmonary nodules of unknown etiology and early stage NSCLC.

Mediastinal endosonography (endobronchial and transesophageal)

Endobronchial ultrasound (EBUS)
Currently, EBUS can be applied in radial and longitudinal techniques (54-56). Radial miniprobe EBUS (R-EBUS) was first described in 1990 (57,58). It utilizes a rotating mechanical transducer (12 to 30 MHz) at the end of a flexible miniprobe which produces a 360 degrees image perpendicular to the longitudinal axis of the catheter. Commonly, the miniprobe is placed through a guide-sheath (8-9 FR) within the working channel of a rigid or flexible bronchoscope. The miniprobe is used to visualize the lesion and to position the guide-sheath which after withdrawal of the miniprobe is used to position instruments for biopsy (e.g., needle, brush, and forceps). R-EBUS is the imaging method with the best detail resolution of the bronchial wall (59) which is of importance for early detection of bronchial carcinoma (60), for differentiating tumor invasion from compression of large airways (61), for assessment of the depth of local tumor infiltration (62,63), and for guidance of endobronchial treatment (photodynamic therapy) in early-stage lung cancer (64). R-EBUS is superior compared to CT in the early T-stages which has been proven in a surgical controlled study. EBUS sensitivity was 89% as compared to CT (25%) and specificity 100% (CT: 80%) (65). R-EBUS may be helpful in the evaluation of unclear stenosis including carcinoma *in situ* which does not infiltrate lamina propria (66,67). An important application of

R-EBUS is biopsy-guidance in peripheral lung lesions (68), in particular of bronchoscopically and fluoroscopically invisible solitary lung nodules (69). A meta-analysis showed a 100% specificity and a 73% sensitivity of R-EBUS-guided biopsy in the diagnosis of peripheral lung cancer (70). The diagnostic yield of R-EBUS-guided biopsy does not exceed CT-guided percutaneous biopsy of solitary lung nodules. However, the major advantage of R-EBUS-guided biopsy over CT-guided biopsy is its superior safety profile, in particular the significantly lower pneumothorax rate (71).

R-EBUS followed by TBNA has also been used for mediastinal lymph node staging of lung cancer (72,73). However, for this indication the longitudinal EBUS (L-EBUS)-technique has prevailed. R-EBUS and L-EBUS are imaging techniques capable of detecting even small mediastinal lymph nodes (66,74,75).

L-EBUS-scopes have been introduced in 2004 (76). They allow ultrasound-guided transbronchial needle biopsy (EBUS-TBNA) which is not possible using radial probes (*Table 2*) (77-80).

L-EBUS [similar to EUS (81)] can be combined with ultrasound technology including strain imaging techniques [real time elastography (RTE)] (77,82-86) and contrast enhanced Doppler techniques (77). Real-time EBUS-TBNA has been shown to have a higher diagnostic yield in mediastinal staging than blind TBNA and has similar sensitivity to mediastinoscopy (5,87,88).

The examination techniques using radial and linear probes have been described in current textbooks (77). The EBUS technique and the key anatomical landmarks are described in detail later in this review.

Endoscopic (transesophageal) ultrasound (EUS)
Conventional EUS via the transesophageal approach is

Table 2 Established equipment for longitudinal EBUS (77)

Equipment	Diameter (mm)	Working channel (mm)	Length (mm)	Field of view	Depth penetration (mm)	Frequency (MHz)	Scan modus	Comment
EB1970UK (video EBUS) [Pentax]	6.9	2.0	600	100°/45° oblique optic	0-120	5/6.5/7.5/9/10	Electronic 75° convex array	Compatible with Hitachi Hi-Vision Scanner
BF-UC180F (EBUS) [Olympus]	6.3	2.2	600	80°/135° oblique hybrid optic	2-50	5/6/7.5/10/12 (EU-ME1); 7.5 (EU-C60); 5/7.5/10/12 (Aloka ultrasound systems)	Electronic 50° convex array	Compatible with EU-ME1, EU-C60 and Aloka ultrasound systems
EB-530US (video EBUS) [Fujinon]	6.3	2.0	610	120°/10° oblique optic	3-100	5/7.5/10/12	Electronic 60° convex array	Compatible with SU-7000 and SU-8000

EBUS, endobronchial ultrasound.
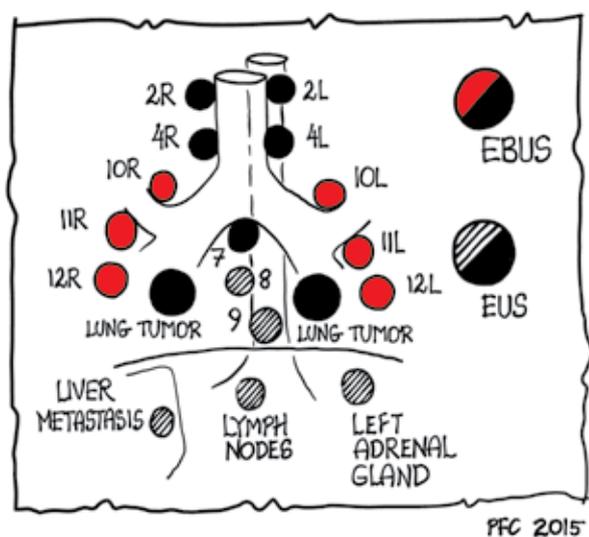
Figure 1 Diagnostic reach of mediastinal endosonography (only EBUS: red dots; only EUS: striped dots; EBUS and/or EUS: black dots). EBUS, endobronchial ultrasound; EUS, endoscopic ultrasound.

a minimally invasive diagnostic and also therapeutically valuable technique. EUS also allows the guidance of biopsies to obtain tissue samples from mediastinal lymph nodes and other mediastinal masses but also from centrally located lung tumors and inflammatory diseases including sarcoidosis and tuberculosis. Currently published data have shown that EUS is a valuable technique for the diagnosis of lung cancer and has improved lymph node staging (89). EUS and EBUS allow lymph node biopsy (90-98).

EBUS and EUS: a complimentary approach

For the evaluation of mediastinal lesions, EBUS and EUS are complimentary methods as various mediastinal and hilar nodal stations can be reached (*Figure 1*) (99). The added value of EUS to EBUS can be summarized by the complementary diagnostic reach of the lower mediastinum and aorto-pulmonary window in selected cases and the evaluation of the left adrenal gland and other infradiaphragmal metastatic sites [*Table 3*, data from: (5,16,100-106)].

EUS is better tolerated by patients compared to EBUS (no coughing or dyspnea). This specifically applies to nodal regions that can be reached by both techniques, the left paratracheal region (station 4 L) and the often affected subcarinal region (station 7). The implementation of endosonographic techniques in lung cancer staging algorithms has also reduced the need for surgical staging options (e.g., mediastinoscopy, thoracoscopy, thoracotomies). However, in the case of suspected nodes by CT/PET imaging and tumor negative findings at EBUS/EUS, additional surgical staging is indicated for optimal nodal staging. This knowledge has gained recognition in recent guidelines (1,2,5,13,107).

Both EUS and EBUS have also been successfully used for the assessment of mediastinal tumor spread of patients with extra-thoracic neoplastic diseases (108-111) and for the evaluation of mediastinal lymphadenopathy of unknown origin and especially for the diagnosis and differentiation of mediastinal granulomatous disease and malignant lymphoma (110,112-124). The examination technique using longitudinal probes has been described in current textbooks (54-56). For a practical approach we refer to the training chapter at the end of this review. The description of currently available

Table 3 Yield and safety of endosonographic methods (EUS-FNA and EBUS-TBNA) to access mediastinal and hilar lymph node stations [data from: (5,16,100-106)]

Diagnostic yield and safety	EUS-FNA	EBUS-TBNA	EUS-FNA + EBUS-TBNA
Accessible LN stations	2 L, (2 R[a]), 3 p, 4 L, (4 R), (5[b]), (6[c]), 7-9, (10 L/R[a]), infradiaphragmatic sites of potential distant metastases (left adrenal, left liver lobe, celiac lymph nodes)	2 L/R, 3, 4 L/R, 7, 10-11 L/R	2-4 L/R, (5[b]), (6[c]), 7-9, 10-11 L/R
No access to mediastinal LN stations	3[a], 11-14	5, 6, 8, 9, 12-14	12-14
Diagnostic sensitivity	Lung cancer staging: 89% (26 studies, 2,443 patients); mediastinal lymphadenopathy: 88% (32 studies, 2,680 patients)	Lung cancer staging: 89% (26 studies, 2,756 patients); mediastinal lymphadenopathy: 92% (14 studies, 1,658 patients)	Lung cancer staging: 86% (8 studies, 822 patients)
Morbidity	0-2.3%	0-1.2%	0-0.8%
Mortality	0%	0-0.08%	0%

[a], Only partial access to this station; [b], access only in the case of distinctive enlarged lymph nodes; [c], access only by transaortic FNA or in semi blind maneuver with a long trajectory through the proximal esophagus along the left subclavian artery. EUS-FNA, endoscopic ultrasound fine needle aspiration; EBUS-TBNA, endobronchial ultrasound combined with transbronchial needle aspiration; LN, lymph node.

equipment including echo-endoscopes and needles and their use has been summarized in detail (77). Pneumological centers are widespread in the USA but less frequent in Europe, e.g., Germany. Therefore, EUS (gastroenterology) and EBUS (pneumology) might be installed in different departments with no or few interactions. The uncoordinated use of EUS and EBUS has been a weakness in the value and clinical work up of ultrasound techniques. The financially and logistically interesting concept consists of a combined endobronchial and esophageal investigation using a single EBUS-echoendoscope where after an initial endobronchial assessment the EBUS scope is subsequently introduced in the esophagus. The results have been promising with a sensitivity of about 90% in staging of NSCLC (17,18). Increasing evidence shows that mediastinal nodal sampling from the esophagus can be performed with the EBUS scope [endoscopic ultrasound with bronchoscope fine needle aspiration (EUS-B-FNA)]. So complete endosonographic staging [EBUS(-TBNA) + EUS-B(-FNA)] can be achieved by a single EBUS scope (17,21,89,125).

An essential part of endosonography is carefully pathology handling. EUS- and EBUS-guided biopsies allow immunostaining in about 80-90% cases which is of importance for subtyping of NSCLC, differential diagnosis to metastases and mesothelioma and for diagnosis of granulomatous diseases

and lymphoma [*Table 4*, data from (1,126-128)].

Cell block technique and preservation of small core particles for formalin fixation and paraffin embedding have improved the results (129-134). In addition genotyping of adenocarcinoma (molecular staging, e.g., *EGFR* mutation analysis, *EML4-ALK* fusion gene), flow-cytometry, FISH analysis and other cytogenetic methods are possible using material obtained by EUS-FNA and EBUS-TBNA from mediastinal lesions (118,126,129,132,135-138). Complete genotyping of lung cancer was possible in a recent RCT in 85.7% of cases using specimens obtained by EBUS-TBNA. Rapid onsite cytopathological evaluation (ROSE) significantly improved the rate of complete genotyping and reduced the need for additional needle passes and repeat invasive procedures aiming at molecular diagnosis (139). A recent guideline of the World Association for Bronchology and Interventional Pulmonology describes the acquisition and preparation of endosonographic samples for the diagnosis and molecular testing of suspected lung cancer (140).

Safety of mediastinal endosonography

EBUS and EUS are safe techniques (141,142). One study including 965 sheath-guided R-EBUS for the evaluation of peripheral lung nodules reported a 1.3% overall complication

Table 4 Phenotyping and differential diagnosis of NSCLC and other mediastinal lesions by immunostaining [data from (1,126-128)]

Tumor type	TTF-1	CK5/6	p63	CK7	CK20	Specific markers
SCLC	+					Keratin, EMA, Ki67 >50% (chromogranin)
NSCLC—squamous cell carcinoma	−	+	+			High molecular cytokeratins
NSCLC—adenocarcinoma	+	+	+	+	−	B72.3, CEA, BerEP4, PAS
Metastases of extrathoracic adenocarcinoma	−			−		E.g., CDX2, CEA, CA19-9, PSA, HepPar1, …
NET	−			−	+	Chromogranin, synaptophysin
Mesothelioma	−	+				Calretinin, WT-1
Lymphoma	−	−	−	−	−	LCA (CD45), CD3, CD5; CD10, CD15, CD19, CD20-23, CD30, Cyclin D1, bcl-2, bcl-6

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; NET, neuroendocrine tumor.

rate with pneumothorax occurring in 0.8% and pulmonary infections in 0.5% of patients (143). A systematic review of 190 studies (n=16,181 patients) found severe adverse events in 0.14% and minor adverse events in 0.22% of patients undergoing mediastinal EUS-FNA or EBUS-TBNA. The most serious adverse events (0.07%) were infections and tended to occur most often in patients with cystic mediastinal lesions and sarcoidosis. Serious adverse events were reported in 0.3% of EUS-FNA and in 0.05% of EBUS-TBNA (142). A nationwide survey in the Netherlands (89 hospitals with estimated 14,075 EUS-FNA and 2,675 EBUS-TBNA) reported seven cases of procedure-related fatalities (0.04%), all occurring in patients of poor performance status [American Society of Anesthesiologists (ASA) Physical Status Classification score III/IV], and 25 serious adverse events (0.15%, EUS-FNA: 0.16% and EBUS-TBNA: 0.11%). Again, most adverse events were of infectious origin (144).

Safety issues have been also discussed elsewhere (127,145,146).

Transcutaneous mediastinal ultrasound (TMUS)

In addition to the head and neck regions (cervical and supraclavicular nodes), mediastinal ultrasound is also able to detect and to guide sampling of pathological lymph nodes (147,148) and neoplasia (149) in the supra-aortal, prevascular, pericardial, upper and lower located paratracheal region as well as in the aorto-pulmonary window. Studies on mediastinal ultrasound published 20 years ago demonstrated that the suprasternal and parasternal approach, when compared with CT, had a sensitivity of 69-100% for the detection of pathological lymph nodes in the mentioned

mediastinal regions (147-150). TMUS is decisive in the supra-aortal, supraclavicular and head and neck regions indicating N3-respective M1-staging (151).

Mediastinal ultrasound is much less often applied and in most centers rarely used in daily routine. Therefore, the value of TMUS is still controversially discussed. The examination technique has been explained and summarized in review articles (152-155) and in respective textbooks (145,156,157).

Definition of mediastinal regions using TMUS

Definitions for lymph node evaluation are similar to CT, EUS and EBUS-criteria. Required criteria for adequate visualization of the different regions are listed in *Table 5*.

Detection of normal lymph nodes

The diagnostic value of ultrasound of mediastinal regions depends on differences in echogenicity between pathological lymph nodes and adjacent tissue. This led to the belief that, in contrast to CT, TMUS was not able to differentiate normal mediastinal lymph nodes from surrounding tissue, mostly due to lack of differences in echogenicity. However, using high resolution ultrasound and color Doppler imaging, lymph nodes are detectable also in healthy subjects. Therefore, it is of importance that normal lymph nodes can be regularly detected in the right paratracheal region and aorto-pulmonary window (158,159). Occasionally normal lymph nodes are also detectable in the subcarinal region. The lower detection rate in the subcarinal region may be a consequence of the deep location of this region within the mediastinum, and also to artifacts caused by heart movements.

Table 5 Transcutaneous ultrasound (TUS) access to different mediastinal regions. Required adequate visualization of anatomic structures

Region	Required adequate visualization of anatomic structures
Supraortic	Whole aortic arch with all branches and both brachiocephalic veins (suprasternal approach)
Paratracheal	Right brachiocephalic vein, brachiocephalic trunk and ascending aorta, right pulmonary artery (suprasternal approach)
Aorto-pulmonary	Whole aortic arch, pulmonary trunk (suprasternal approach)
Prevascular	Ascending aorta and pulmonary artery (right and left parasternal approach)
Subcarinal	Ascending aorta, right pulmonary artery, left atrium in two planes (right and left parasternal approach)
Pericardial	Right atrium, right and left ventricle, pericardial fat pads bilaterally (right and left parasternal approach)

Table 6 Lymph nodes in the mediastinum detected by ultrasound in 20 human cadavers (158,159)

Mediastinal region	Proportion of cadavers with detectable lymph nodes	Number of lymph nodes (detected by ultrasound)*	Lymph node size (determined by ultrasound) (mm)[#]	Lymph node size (morphometric measurement) (mm)[#]
Paratracheal	17/20	1.9±1.0	11×6	11×6
Aorto-pulmonary window	18/20	1.7±0.7	11×5	11×4

*, The number of lymph nodes is given as mean ± standard deviation; [#], the lymph node size is given as the mean longitudinal diameter × mean transversal diameter.

Mediastinal ultrasound in human corpses

To confirm that normal lymph nodes could be detected by mediastinal ultrasound, 20 human cadavers (11 male, 9 female, 66.4±10.9 years, range: 45-76 years, all without known diseases affecting mediastinal lymph nodes) were examined before and after autopsy to validate the sonographic findings with histologic examinations (159). Lymph nodes were sonographically detected in 85% of the cadavers in the paratracheal region, and in 90% in the aorto-pulmonary window. The longitudinal diameter of detected lymph nodes in the corpses was 8-22 mm in the paratracheal region and 8-17 mm in the aorto-pulmonary window. The sonographically determined lymph node size correlated well with the morphometric measurements of the macro-pathological specimens. In the paratracheal region, 75% of all lymph nodes identified *in situ* after thoracotomy were detected sonographically, whereas in the aorto-pulmonary window 91% of all lymph nodes identified after thoracotomy were also detected sonographically. All normal lymph nodes were oval in shape. No round lymph node was found. In 21% a lymph node sinus could be identified. Histologic examination revealed lymphatic tissue in all sonographically detected lymph nodes (*Table 6*) (158,159).

Mediastinal ultrasound in healthy subjects

In the paratracheal region lymph nodes were detected sonographically in 35% of the healthy subjects, in the aorto-pulmonary window in 45% of the cases and in the subcarinal region in 12.5%. All detected lymph nodes had a hypoechogenic appearance. In contrast, in the supra-aortic, the prevascular and the pericardial regions of the healthy subjects lymph nodes >6 mm were not detected by mediastinal ultrasound (159). This finding is in accordance to the literature (160). In the healthy subjects the longitudinal diameter of detected lymph nodes was 10-19 mm in the paratracheal region and 12-19 mm in the aorto-pulmonary window. Due to its typical location and shape, in the aorto-pulmonary window the superior pericardial recessus always could be differentiated from lymph nodes (159,161).

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Footnote

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An update on measurement and monitoring of cough: what are the important study endpoints?

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Abstract: Considerable progress has been achieved in the development of tools that assess cough. The visual analogue scale (VAS) for cough severity is widely used in clinical practice because it's simple and practical. The Leicester cough questionnaire (LCQ) and the cough-specific quality of life questionnaire (CQLQ) are the most widely used health status questionnaires for adults with chronic cough. They are well validated for assessing the impact of cough. Cough can be assessed objectively with challenge tests that measure the sensitivity of the cough reflex. Cough challenge tests are better used to determine the mechanism of action of therapy, rather than efficacy. Cough frequency monitoring, the preferred tool to objectively assess cough, is increasingly being used as primary end-points in clinical trials. The most widely used cough monitors are the Leicester cough monitor (LCM) and VitaloJak. They are ambulatory devices that consist of a microphone and recording device. Cough frequency monitors do not reflect the intensity or the impact of cough; hence their relationship with subjective measures of cough is weak. Cough should therefore be assessed with a combination of subjective and objective tools. There is a paucity of studies that have investigated the minimal important difference of cough frequency monitors, rendering further investigations needed.

Keywords: Cough; cough intensity; monitoring; measurement; quality of life

Introduction

Cough is associated with significant physical and psychological morbidity (1). The assessment of cough severity is important for evaluating the response to therapy (2,3). The severity of cough can be measured in several aspects: symptom severity, frequency, intensity and impact on quality of life. A number of validated tools are now available to assess cough (*Table 1*). A combined subjective and objective assessment is necessary for comprehensive evaluation (4). This review will focus on the measurement and monitoring in adult patients with chronic cough.

Visual analogue scales (VAS)

VAS are widely used for the subjective assessment of cough because they are brief and easy to use (5), with which the

subject is asked to mark on a 100 mm scale between 'no cough' and 'the worst cough severity'. The advantage of VAS is that they assess the symptom in isolation and reflect the severity. They are also freely available. The VAS is practical for use in research. However, it is still lacks published data reporting its validity and the minimal important difference (MID). The VAS has also been evaluated in acute and sub-acute cough (6,7). The MID has been reported to be 17 mm in acute cough (6). The MID for VAS in chronic cough is likely to be similar to that of acute cough in the authors' opinion but this has not be studied. The VAS is highly responsive to change (8). In the authors' opinion, the use of VAS should be encouraged because it is familiar to clinicians, brief and clinically meaningful. Furthermore, similar tools are used effectively in the management of other symptoms such as

Table 1 Cough measurement tools
Symptoms
Visual analogue scales (VAS)
Cough severity score (CSS)
Cough severity diary (CSD)
Health related quality of life (HRQOL)
Leicester cough questionnaire (LCQ)
Cough-specific quality of life questionnaire (CQLQ)
Objective
Cough reflex sensitivity
Capsaicin
Citric acid
Fog
Tartaric acid
Cough monitors
Leicester cough monitor (LCM)
VitaloJak

chest pain. The benefits of using the VAS include improved communication between clinicians regarding the severity of cough and documenting longitudinal observations. The VAS can also be used to assess the urge, frequency and intensity of cough.

Health related quality of life (HRQOL)

Cough can have a wide-ranging impact on the patient, and is very disruptive. It can lead to physical symptoms such as syncope, chest pain, urine incontinence, vomiting, headache, and sleep disturbance.

It is associated with psychological morbidity such as anxiety and depression and socially it can lead to embarrassment and disruption of activities. HRQOL can be quantified by using specifically designed questionnaires. Their advantage in comparison to VAS scales is that they capture the wider impact of cough on the individual. HRQOL questionnaires provide a structured and standardised approach to quantifying health status. They are well validated for this purpose and highly responsive to change (9). HRQOL questionnaires can be categorised into generic tools, such as the Short Form-36 (SF-36) or disease specific. A limitation of generic tools is that they are generally longer questionnaires and potentially less responsive to change. It is advisable to use cough-specific HRQOL questionnaires for the assessment of health status

in patients with cough. The two most widely used HRQOL questionnaires for adult patients with chronic cough are the Leicester cough questionnaire (LCQ) and cough-specific quality of life (CQLQ) (10,11). For children, a recently validated questionnaire is now available: the paediatric cough-quality of life questionnaire (PC-QLQ) (12).

Leicester cough questionnaire (LCQ)

The LCQ is a 19-item questionnaire comprising three health domains: physical, psychological and social (10). It is brief, easy to use and score. It was developed using a patient-rated importance scale, also known as a clinimetric method, for patients with chronic cough but has also been validated for patients with chronic obstructive pulmonary disease (COPD), bronchiectasis and acute cough (13-15). It was translated into a wide range of languages and it has been the most widely used of all cough HRQOL questionnaires since 2001. The LCQ is well validated with very good internal reliability, repeatability and responsiveness (10). The MID in acute and chronic cough are 2.0 and 1.3 respectively (6,15). The LCQ has been used in clinical trials of Erythromycin, Gabapentin, cough suppression physiotherapy and Interferon therapy. It is currently being used in a clinical trial of Transient Receptor Potential Ankyroid Receptor Type 1 inhibitor.

Cough-specific quality of life questionnaire (CQLQ)

The CQLQ, developed by clinimetric methodology, is a 28-item questionnaire with 6 domains developed in the US (11). The CQLQ is well validated in chronic and acute cough. It has good internal reliability, repeatability, responsiveness and the MID in chronic cough is 13 units (16). It has recently been used in a clinical trial of Esomeprazole in chronic cough and Thalidomide in patients with cough associated with idiopathic pulmonary fibrosis (17,18).

Other subjective questionnaires

Cough severity score (CSS)

The CSS is a two-part questionnaire referring to symptoms during the day and night time (19). The response scale captures cough frequency, intensity and overall impact. There is little clinical experience with this tool and the MID has not been reported. Further studies of this tool are underway.

Cough severity diary (CSD)

CSD is a brief tool, comprising seven items (20). It was developed using feedback from patients. In addition to severity, it captures the impact of cough intensity. There is, however, little clinical experience with this tool. The MID has not been studied.

Objective assessment of cough

There has been significant progress recently in the development of objective tools to assess cough. The clinical use of objective tools is to validate the presence of cough in subjects and evaluate the improvement following therapy. Until the development of cough monitors, assessment of cough reflex sensitivity was the only objective method being used. The limitation of cough reflex sensitivity measurement is that it only assesses the mechanism of cough, not the efficacy from the patient's perspective. The recent technological advances in recording devices have led to significant achievements in the field of cough detection monitoring. There is general consensus that the assessment of cough frequency is the gold-standard objective tool (21). It is also possible to measure cough intensity with physiological measures, but they are invasive, and not practical for the clinical setting.

Cough reflex sensitivity—the cough challenge test

The methodology to measure the sensitivity of the cough reflex is, in principle, similar to that used to assess bronchial responsiveness with agents such as methacholine. Cough is provoked by the inhalation of nebulised tussive agents, usually capsaicin or citric acid. Other valid tussive agents include tartaric acid, fog, cinnamaldehyde and bradykinin. The test result is usually expressed as the concentration of tussive agent that causes two or five coughs (C2 or C5). Cough reflex sensitivity assessment is reproducible and responsive in patients (22). It is frequently used in both animal and human research studies. A major limitation to its use in clinical practice is its inability to discriminate patients with cough from healthy subjects (22). Another limitation is that capsaicin dilutions need to be made frequently due to its instability in solution in contrast to other challenge tests such as methacholine. The test also needs to be performed in a ventilated room. The utility of cough reflex sensitivity tests in clinical trials is subsiding because they do not consistently reflect the efficacy of therapy from the patient's perspective. Its future use might be limited to researchers studying the mechanism of action of anti-tussive therapy. It may be particularly useful in drug development when specific cough reflex pathways can be investigated. The future challenge lies in developing methodology that can reliably discriminate healthy subjects from those with cough, and this may be possible by using higher concentrations of tussive agents than currently used (23).

Cough frequency monitors

Cough frequency assessment is considered the gold standard for the objective assessment of cough. Ten years ago, the development of cough monitors was limited by the recording capacity of tape recorders and poor battery life. The development of MP3 recorders overcame hardware limitations, and therefore the focus turned to the development of software for automated cough detection. There has been significant progress in the development of automated cough detection software, but with mixed results. Many cough monitors have insufficient accuracy for cough detection, and therefore their use is limited or they are not used at all, such as the Hull Automated Cough Monitor, LifeShirt and Pulmotrack (24-26). A particular challenge has been the discrimination of cough sounds from speech and other noise. Two cough monitoring systems have demonstrated good validity and are being used more widely in clinical trials, the Leicester cough monitor (LCM) and the VitaloJak. They differ in their approach to cough detection; the VitaloJak requires manual assessment of condensed cough recordings, and the LCM is largely automated.

VitaloJak

The VitaloJak consists of two microphones (contact and free-field) and an MP3 recorder (27). A condensed version of the recording is assessed manually to listen for cough sounds. The condensed 24-hour cough recording is on average 1.5 hours long. The accuracy of this monitor is dependent on the observer conducting the manual counting and, in experienced hands, it is very good (27). A limitation of manual assessment is that it is labour-intensive and time-consuming. In *Table 2*, we compare the characteristics of the VitaloJak and LCM. The characteristics are similar, despite the very different approaches to cough detection.

Table 2 A comparison of the characteristics of LCM *vs.* VitaloJak

Characteristics	LCM	VitaloJak
Hardware	External microphone and MP3 recorder	External microphone and MP3 recorder
Recording duration (hours)	24+	24
Analysis method	Automated software	Manual/condensed recording
Operator input time (mean time per 24-hour recording) (min) (6,27)	5	87
Association with subjective measure (HRQOL, LCQ) (correlation coefficient) (28,29)	−0.60	−0.62
Cough frequency (coughs per hour)		
Healthy subjects (30,31)	1	1
Chronic cough (30,32)	20	17
Acute cough URTI (6,33)	15	12
COPD (30,31)	9	9

Cough frequency recordings per 24-hours. LCM, Leicester cough monitor; LCQ, Leicester cough questionnaire (patients with chronic cough); HRQOL, health related quality of life; COPD, chronic obstructive pulmonary disease; URTI, upper respiratory tract infection.
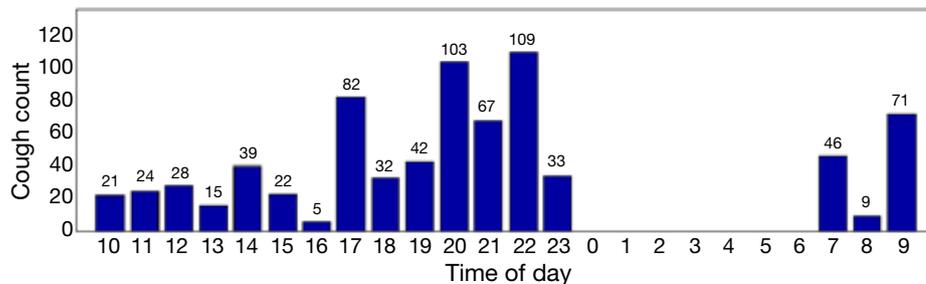
Figure 1 A 24-hour cough frequency in a patient with chronic cough.

Leicester cough monitor (LCM)

The LCM comprises of a free-field microphone and an MP3 recorder (34). Cough detection is largely automated using specifically designed software. This involves minor refinement by an operator (5 minutes per 24-hour recording). The sensitivity and specificity for cough detection is very good (30,34). The LCM has been used in single and multi-centre clinical trials (35,36).

The relationship between objective cough frequency and subjective measures of cough

The relationship between objective cough frequency and subjective measures of cough such as VAS and HRQOL is mild to moderate (28). This reflects the different aspects of cough assessed by these tools such as perception *vs.* actual frequency. The poor relationship does not imply that cough

frequency monitoring is inaccurate for the detection of cough. The accuracy of automated cough monitors is established by comparison to manually counted recordings (34).

Cough frequency monitors in the clinical setting

Cough frequency monitors are now sufficiently practical for use in the clinic and clinical trials. The automation of cough monitoring has facilitated this. Patients with chronic cough on average cough every two minutes in a 24-hour period (*Figure 1*). This contrasts with healthy subjects who cough on average every thirty minutes. Cough has a diurnal pattern in both disease and health states; cough is significantly reduced at night. In patients complaining of cough, the pattern and frequency of cough is very similar irrespective of underlying causes such as gastro-oesophageal reflux and cough variant asthma. In the authors' experience, the frequency of cough

events in patients with chronic lung diseases such as asthma, COPD and idiopathic pulmonary fibrosis depends on the prominence of cough as a symptom. In some patients, the frequency can be as high as those with idiopathic chronic cough. Cough monitors are the best tools to discriminate patients with cough from healthy subjects.

The utility of cough monitor in clinical practice has not been established. The severity of cough can simply be assessed by asking the patient, but the disadvantage of this is that some patients and clinicians may be poor judges of symptom severity. Cough monitors can potentially be used to validate the presence of cough. They can also be used to quantify the response to therapy. They have been used in the research setting to assess the temporal relationship of cough with episodes of gastro-oesophageal reflux; the clinical usefulness of this has not been established (37). The benefits of cough monitoring technology in the clinic need further investigation. Cough monitors however are considered an important end-point in clinical trials. The strength of cough monitors is their objectivity and that they can discriminate healthy subjects from those without cough. This may be useful for selecting patients for clinical trials. Cough frequency has been reported to be a repeatable measure in patients with stable chronic cough. In contrast, in acute cough, cough frequency is not repeatable due to natural recovery (6). The rate of improvement in cough frequency may be a better measure in such patients.

Cough frequency monitors as a study endpoint

Cough frequency can be expressed by a range of outcome measures. For example, absolute counts *vs.* coughs per hour. Cough can be assessed during daytime, night time or 24 hours. It is perhaps better to express change in cough frequency as a percentage or fold change, rather than absolute change, since it has a wide range. An advantage of using cough frequency measures to determine the sample size of studies is that considerably fewer subjects are required in comparison to subjective outcome measures, such as HRQOL. This is due to the comparatively larger change in cough frequency required to demonstrate a MID (6). For example, in acute cough, the MID for cough frequency has been reported as a 54% reduction (6).

Cough intensity

The intensity of cough may also be relevant to the impact

on an individual, in addition to the frequency. Little is known about cough intensity and its importance to patients. It can be assessed subjectively with VAS or objectively with physiological measures such as cough flow, oesophageal pressure or electromyography. Flow is the most practical physiological measure since it is non-invasive and relatively easy to perform. However, its limitation is that it is not ideal for continuous monitoring in an ambulatory setting. It may be possible to assess cough intensity with sound (38). Further studies are needed to determine whether cough sound is a valid measure of cough intensity, its relationship with subjective measures and clinical relevance.

Conclusions

A number of tools are now available to assess cough. It is likely that a combination of subjective and objective assessment is necessary to assess cough comprehensively since each tool assesses very different aspects. For subjective assessment, the VAS is ideal for use in the clinic since it is practical, and it can be used to communicate the severity of cough to other clinicians and for longitudinal observation. It is also good for use in clinical trials. The VAS should be complemented by HRQOL assessment to assess impact. HRQOL questionnaires for cough are the most validated of all cough assessment tools. The LCQ and the CQLQ are the most widely used for adult patients with chronic cough. It is arguable that the primary outcome measure of clinical trials should be objective, and cough frequency monitors are best placed for this. Cough frequency monitors are increasingly being used in clinical trials. The clinical experience of using cough monitors to date is that they are practical and valid. They should always be complemented by assessment of HRQOL since a reduction in objective cough frequency without subjective improvement would not be considered clinically important. Further work is necessary in a number of areas to improve the utility of cough assessment tools. The investigation of the MID and more precise sample size estimations are good examples. The assessment of cough in chronic lung disease such as COPD and idiopathic pulmonary fibrosis is likely to be very fruitful.

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Effect of viral upper respiratory tract infection on cough reflex sensitivity

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Abstract: Acute viral upper respiratory tract infection (URI; common cold) is among the most common medical conditions affecting man, with cough being a typical feature of the associated syndrome. Studies employing capsaicin inhalation challenge to measure cough reflex sensitivity have demonstrated a transient tussive hyperresponsiveness induced by URI that reverts to normal by 4-8 weeks post infection. Mechanisms proposed to explain the induction of cough by URI include a number of infection-associated airway effects, such as enhanced release of cytokines, neurotransmitters, and leukotrienes; increased neural receptor levels; reduced activity of neutral endopeptidases; transient modulation of afferent neural activity; mucus hypersecretion; and, possibly, effects on cholinergic motor pathways. Recent studies evaluating urge-to-cough (UTC), the sensation of irritation preceding the motor act of coughing, have demonstrated that URI induces a transient enhancement of UTC analogous to the effect observed on cough reflex sensitivity. The recently introduced concept of the Cough Hypersensitivity Syndrome may provide an explanation for the commonly observed clinical phenomenon of acute viral URI triggering what will develop into chronic, refractory cough in a subgroup of patients.

Keywords: Cough; capsaicin; common cold; viral infection; virus

Introduction

Cough is among the most common reasons for which patients worldwide seek medical attention (1). Most cases of acute and subacute cough are due to acute viral upper respiratory tract infections (URI), otherwise known as the common cold. Given the sheer enormity of the problem of common cold worldwide, the medical and economic implications of cough due to this condition are of tremendous significance. Not only does acute cough due to URI result in tremendous financial expenditure, but the available treatments for acute cough are limited by lack of efficacy or, as in the case of opiates, for example, intolerable side effects at antitussive doses (2,3).

Measurement of cough reflex sensitivity

Measurement of cough reflex sensitivity has allowed insight into the effects of viral URI on human cough reflex sensitivity. Among the various provocative agents used for induction of cough in the laboratory, capsaicin, the pungent extract of red chili peppers, has gained favor as the tussive agent of choice, given its ability to induce cough in a safe (4), dose-dependent and reproducible manner (5,6). The typical end points of capsaicin inhalation challenge are C_2 and C_5, the concentrations of capsaicin inducing two or more, and five or more coughs, respectively. *Figure 1* illustrates a typical dose-response curve generated during a capsaicin challenge study.

Effect of viral URI on cough reflex sensitivity

Employing capsaicin inhalation challenge to measure cough reflex sensitivity, O'Connell and colleagues initially demonstrated that cough reflex sensitivity is transiently enhanced in otherwise healthy subjects during acute viral URI, compared with the post-recovery state (7).
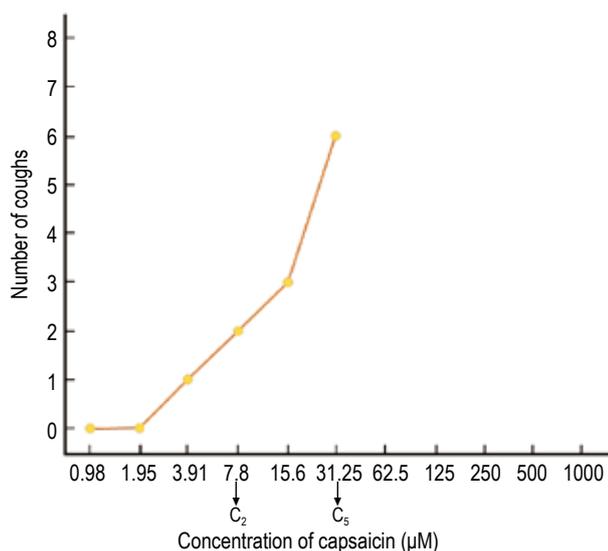
Figure 1 Typical dose-response curve observed during capsaicin inhalation challenge. In this subject, C_2, the concentration of capsaicin inducing two or more coughs, is 7.8 μM; C_5, the concentration of capsaicin inducing five or more coughs, is 31.25 μM.
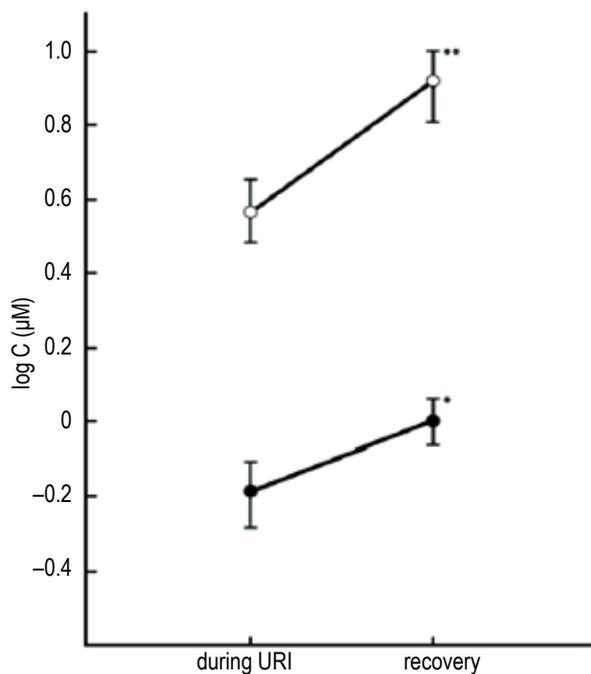
Figure 2 Mean values for log C_5 (open circles) and log C_u (solid circles) during viral upper respiratory tract infection (URI) and after recovery (4-8 weeks after URI). Error bars indicate ±SEM. Values were significantly lower (i.e., sensitivity enhanced) during URI. *P=0.046; **P=0.001. C_5 was affected to a greater degree by URI than was C_u (P=0.044). [from Reference (8), with permission].
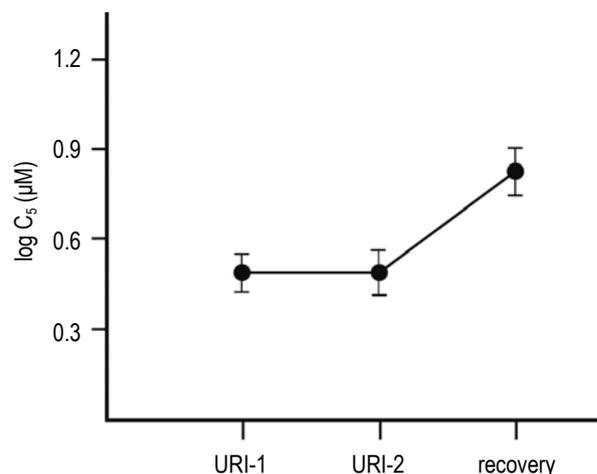
Figure 3 Mean log C_5 values for subjects (n=42) measured on consecutive days during the first 8 days of acute viral upper respiratory tract infection (URI-1 and URI-2), and 4-8 weeks after URI (recovery). Error bars indicate ±SEM.

These findings were subsequently confirmed in a similar population (8), as shown in *Figure 2*, with C_5 significantly decreased (cough reflex sensitivity enhanced) during URI compared with repeated measurement after recovery (4-8 weeks post-URI).

A recent study was the first to perform multiple cough reflex sensitivity measurements during the acute phase of viral URI in otherwise healthy subjects (9). Capsaicin cough challenge, performed twice during the first eight days of acute URI, demonstrated stability of cough reflex sensitivity during acute URI, with subsequent diminution (increased C_5) post-recovery (*Figure 3*). This observation is of significance to future investigators evaluating the effect of a pharmacological intervention on acute cough, as changes in cough reflex sensitivity can be assumed to reflect the effect of the study drug, rather than significant, naturally occurring fluctuations in cough reflex sensitivity occurring during the early stages of acute URI.

Relatively few studies have evaluated the effect of a drug on cough reflex sensitivity during URI. The widely used expectorant, guaifenesin, has been shown in two clinical trials to inhibit cough reflex sensitivity in subjects with acute viral URI, but not in healthy volunteers (10,11). Similarly, the anticholinergic bronchodilator, tiotropium, has been shown to suppress cough reflex sensitivity in otherwise healthy nonsmokers with acute URI, but not in healthy controls (12). Interestingly, the antitussive effect of tiotropium occurred without demonstrable bronchodilation, thus highlighting the concept that tiotropium may

Table 1 Proposed mechanisms of virus-induced cough*

Effects on airway epithelium - inflammation and cytokine release (IL-1, TNF-α, IL-6, IL-8, GRO-α, IL-11, RANTES, GM-CSF, eotaxin)
Increased airway neurotransmitter levels - Substance P;
Reduced activity of neutral endopeptidases in airway;
Increased neural receptor levels (NK-1);
Modulation of afferent neural activity (transient).
Effect on cholinergic motor pathways (bronchoconstriction, airway hyperresponsiveness)
Increased leukotriene production;
Mucus hypersecretion.
*Adapted from Reference (15).

have pharmacological effects beyond that of reversal of pathological airway obstruction (13). Notably, both of these agents inhibited capsaicin sensitivity in subjects with URI, whose cough reflex sensitivity was transiently enhanced, whereas the drugs had no effect in healthy volunteers, whose cough reflex was at its baseline. These observations raise the concept that subjects with pathologically enhanced cough reflex sensitivity, rather than healthy volunteers, comprise the optimal study population for clinical trials evaluating potential antitussive agents (14).

Mechanisms of enhanced cough reflex sensitivity during viral URI

Numerous mechanisms have been proposed to explain the transient cough and enhancement of cough reflex sensitivity associated with acute viral URI (15). Direct effects of the viral infection on airway epithelium include inflammation and cytokine release. Other airway effects of URI include increase in neurotransmitter levels, such as Substance P; reduced activity of neutral endopeptidases; increased neural receptor levels (NK-1); and, transient modulation of airway neural activity. Increased leukotriene production and mucus hypersecretion are likely additional contributors to cough induction. Viral infections induce bronchoconstriction and airway hyperresponsiveness through their influence on cholinergic pathways, but the significance of these effects on cough and cough reflex sensitivity remains to be elucidated (*Table 1*).

It is of interest, and likely quite significant, that many patients presenting with chronic cough relate the onset of their longstanding condition to an episode of URI. The newly emerging concept of the Cough Hypersensitivity Syndrome (16-19) suggests that an underlying hypersensitivity of the cough reflex potentiates the effect of an exogenous stimulus

such as acute viral URI, resulting in refractory, chronic cough in a particular subgroup of individuals whereas the same stimulus causes a merely transient cough in the great majority of the population.

Effect of viral URI on the urge-to-cough (UTC) sensation

Recently, increasing interest has focused on the sensation of irritation that precedes the motor act of coughing; this phenomenon has been termed the UTC (20-23). Studies employing functional magnetic resonance imaging in subjects administered inhaled capsaicin have demonstrated that the UTC sensation is associated with activations in a variety of cortical brain regions (24,25). As is the case with the motor cough reflex, acute viral URI has been demonstrated to induce a transient enhancement of the UTC sensation (8). As shown in *Figure 2*, UTC, as measured by C_u, the lowest concentration of capsaicin inducing the UTC sensation without an associated motor cough (23), is significantly enhanced during URI compared to the post-recovery state.

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Recent additions in the treatment of cough

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Background: Worldwide, cough is regarded as a challenging clinical problem due to its frequency and often limited therapeutic options. Chronic cough that remains refractory to usual medical treatment causes significant quality of life impairment in people with this problem.

Methods: We have examined current evidence on recent additions in the treatment of cough, specifically treatment of refractory chronic cough with speech pathology and gabapentin. Relevant randomised control trials, reviews and case reports were identified through a PubMed and SCOPUS search of English-language literature referring to these concepts over the last eight years.

Summary: Of the one hundred and two articles comprising this review the majority investigated the role of the transient receptor potential (TRP) receptors TRP Vanilloid 1 (TRPV1) and TRPA1 in cough and the potential of TRP antagonists as effective anti-tussives. However, these have only been tested in the laboratory and therefore their clinical effectiveness is unknown. Behavioural treatments such as speech pathology have gained momentum and this was evident in the increasing number of articles investigating its positive effect on cough. Investigation on the effectiveness of neuromodulating medications in the treatment of cough have been supported primarily through case series reports and prospective reviews however; their use (particularly gabapentin) has been significantly advanced through recently conducted randomised controlled trials.

Conclusions: Recent additions in the treatment of chronic cough have been significant as they consider cough to have a unifying diagnosis of cough hypersensitivity with or without the presence of a neuropathic basis. Primarily, effective treatments for chronic cough target these areas and include behavioural treatment such as speech pathology and pharmaceutical treatment with neuromodulating medications such as gabapentin.

Keywords: Chronic cough; speech pathology; gabapentin; neuropathic cough; cough hypersensitivity

Background

Cough is the most commonly reported symptom during primary care consultations and chronic cough is the most common reason for patients seeking specialist respiratory assessment. Refractory cough accounts for up to 40% of these cases and remains challenging for the clinician as there are limited effective treatment options. There is also a profound effect on the patient as a prolonged cough has significant impact on their quality of life. Fortunately, recent advances have been made in the treatment of refractory and idiopathic chronic cough and even more fortuitous is that these include both advances in pharmacological and non-pharmacological treatments for patients.

These new treatments have originated from a number of different sources including otolaryngology where there is recognition of the upper airway involvement in cough; proximity of the larynx/pharynx to the oesophagus and the upper and lower respiratory tract; and hypersensitivity of the larynx from upper and lower airway inputs such as respiratory tract infections and laryngopharyngeal reflux.

Up to 40% of people with refractory chronic cough suffer significant voice problems (1) and around 56% may also have paradoxical vocal cord movement (PVCM) that is, adduction of the vocal cords during inspiration and sometimes expiration (2). Speech pathologists and physiotherapists have previously recognised and demonstrated the effectiveness of treating laryngeal symptoms and breathing disorders through tailored interventions for individuals with persistent cough (3-6).

Traditionally, chronic cough has been suggested to be due to three conditions, asthma, post nasal drip, and gastroesophageal reflux disease. A different paradigm has now been proposed in which cough is viewed as the primary condition characterised by afferent neuronal hypersensitivity and different aspects of this syndrome are manifest in the different phenotypes of cough. There are several advantages to viewing "cough hypersensitivity" (7) as the unifying diagnosis; communication with patients is aided, aetiology is not restricted and therapeutic avenues are opened.

Sensory laryngeal neuropathic cough shares similarities to other hypersensitivity neuropathic syndromes such as chronic pain. Central neural mechanisms are thought to be involved since refractory chronic cough patients have abnormal laryngeal sensations (laryngeal paraesthesia), increased sensation to non-tussive stimuli (allotussia), and a heightened response to tussive stimuli (hypertussia) (8) that responds to centrally acting medications such as gabapentin (9). The pathophysiological basis of this hypersensitivity in the larynx involves upregulation of the transient receptor potential (TRP) nociceptors. Capsaicin hypersensitivity can be mediated through TRP Vanilloid 1 (TRPV1), and airway epithelium immunostained with anti-TRPV1 antibody has shown that patients with chronic cough of diverse causes have a five-fold elevation of TRPV1 containing nerves (10). Novel compounds are in development to block these receptors and hold promise for chronic cough and upper airway hypersensitivity. As these compounds await clinical testing this review will primarily focus on speech pathology management and gabapentin treatments for chronic cough.

Methods

Search process

We have examined current evidence on recent additions in the treatment of cough, specifically treatment of refractory chronic cough with speech pathology and gabapentin. Relevant randomised control trials, reviews and case reports were identified through a PubMed and SCOPUS search of English-language literature referring to these concepts over the last eight years. One hundred and twenty six articles containing the keywords of 'chronic cough' with 'treatment', and/or 'speech pathology/therapy', 'gabapentin', 'pregabalin', 'TRP antagonists' were identified. Articles identified as not including treatment of the cough specifically or referring to a paediatric population were removed resulting in one hundred and two relevant articles.

Summary

Of the 102 articles, 12 relevant review articles dedicated to the treatment of chronic cough with either mainstay diagnostic therapy combined with speech therapy (11), empiric therapy (12,13), speech pathology/behavioural techniques (14-17) or with pregabalin or gabapentin were identified (18-22). A further 20 articles focused on the investigation of the TRP channels (TRPV1 and TRPA1) (23-42) as potential treatment targets for respiratory disease with cough. Ten investigated the treatment of cough with speech pathology alone (3-5,43-49). Five articles investigated the role of gabapentin (9,50-53) in the treatment of cough, one article focused on pregabalin treatment of cough (54) and a further two articles included both pregabalin and gabapentin treatment trials (55,56). Amitriptyline a tricyclic antidepressant was also investigated in the treatment of chronic cough in two recent reports (57,58). Four studies investigated a multifaceted treatment scenario such as with cause-directed therapy with speech pathology or neuromodulating treatment (59-62). Remaining articles investigated other treatments for cough such as with opiates (63,64), itraconazole (65), corticosteroids (66,67), proton pump inhibitors (68-71), antihistamines (72), and even camphor (73). This reference list is not exhaustive as the primary focus of this review is to provide a summary of recently investigated clinical treatments for chronic cough specifically the treatment of refractory chronic cough with speech pathology and gabapentin.

Results

Non-pharmacological/behavioural approaches to cough

Non-pharmacological approaches such as speech pathology

Table 1 Comparison of mean (SD) pre- and post-intervention breathing, cough and upper airway symptom scores and degree of change for participants in the treatment and placebo groups

Symptom	Group	Pre	Post	Difference	95% CI	P value
Breathing	Treatment[†]	7.9 (4.1)	5.0 (4.2)	2.9 (3.6)	1.8 to 3.9	<0.001*
	Placebo[†]	6.6 (4.7)	5.5 (3.5)	1.1 (3.4)	0.1 to 2.0	0.004*
	Difference[‡]			2.2 (3.7)	0.4 to 3.2	<0.001*
Cough	Treatment[†]	8.8 (2.8)	4.9 (3.0)	3.9 (3.2)	3.0 to 4.9	<0.001*
	Placebo[†]	7.5 (3.6)	6.3 (3.5)	1.2 (3.4)	0.3 to 2.2	<0.001*
	Difference[‡]			2.8 (3.6)	1.3 to 4.0	0.003*
Upper airway	Treatment[†]	9.2 (6.6)	6.5 (6.3)	2.7 (4.7)	1.4 to 4.1	<0.001*
	Placebo[†]	7.4 (4.9)	7.4 (5.5)	0.1 (4.1)	–1.1 to 1.2	0.946
	Difference[‡]				1.5 (4.8)	0.9 to 4.4	0.002*

[†], calculated using Wilcoxon signed rank test; [‡], calculated using Mann-Whitney U test; *, statistically significant difference between pre and post measures and difference between the two comparison groups of treatment and placebo are statistically significant also.

focus on reducing laryngeal hypersensitivity. As far back as 1987 Gay et al. (74) utilised a treatment program involving speech therapy, relaxation and psychotherapy in subjects with psychogenic habit cough.

A year later Blager, Gay and Wood (75) reported a similar treatment program and applied techniques such as diaphragmatic breathing, laryngeal tension reduction and psychotherapy.

Recognition of this treatment for cough really began after Vertigan published the largest ever randomised controlled trial on speech pathology management of refractory chronic cough in 2006 (47). The aim of this trial was to determine the efficacy of a speech pathology intervention programme for chronic cough. Patients were randomly allocated to receive either the speech pathology programme (termed SPEICH-C) which comprised four components on (I) education about the nature of chronic cough; (II) strategies to control the cough; (III) psycho-educational counselling; and (IV) vocal hygiene education to reduce laryngeal irritation, or to a placebo intervention that involved lifestyle changes. Both of these interventions involved the patient spending four clinic sessions with a qualified speech pathologist.

The magnitude of improvement was significantly greater in the treatment group than in the placebo group for all symptom scores (*Table 1*, breathing, cough and upper airway symptoms only shown here). A lack of significant difference in the pre-intervention symptom scores between these groups indicated that improvements observed in the treatment group were due to the intervention.

This study showed that speech pathology treatment

based on the approaches used in vocal cord dysfunction/PVCM and hyperfunctional voice disorders is also effective in chronic cough. Although this study really informed investigators of the potential of this treatment for chronic cough the mechanism behind the symptom improvement had not been determined nor were the effects of the treatment on laryngeal dysfunction known.

Ryan *et al.* therefore investigated the relationship between cough reflex sensitivity and laryngeal dysfunction, which was assessed as PVCM and extrathoracic airway hyperresponsiveness (EAHR), in patients with chronic cough. Adults with chronic cough and healthy controls were assessed with validated subjective and objective cough outcomes and fiberoptic laryngoscopy to identify PVCM.

PVCM was found to be present in 56% of participants with chronic cough, accompanied by cough reflex hypersensitivity, and impaired quality of life (measured with the Leicester Cough Questionnaire, LCQ).

Inspiratory airflows were reduced in the Cough with PVCM subjects, and there was significant EAHR.

This study identified that laryngeal dysfunction commonly occurs in chronic cough where it causes laryngeal symptoms, quality of life impairment and PVCM. It is accompanied by hyperresponsiveness of the extrathoracic airway to inhaled stimuli that lead to reduced inspiratory airflow. PVCM and EAHR were also found to be associated with cough reflex hypersensitivity and after implementing a cough diagnostic and treatment algorithm (76) with speech pathology treatment (47), the degree of improvement in cough reflex sensitivity correlated with the improvement in EAHR (61).

These findings provided an explanation for the

Table 2 Mean change in cough outcome measures from pre-treatment (baseline) to full-dose treatment for participants on placebo compared to participants on gabapentin

Outcome	Placebo mean change from baseline to treatment period[^]	Gabapentin mean change from baseline to treatment period[^]	Difference between treatment groups (Gabapentin *vs.* Placebo) during the treatment period* Coefficient (95% CI)	P-value
LCQ[#]	+1.1	+2.5	1.80 (0.56, 3.04)	0.004
Cough frequency, (coughs/h)[§]	−4.3	−22.5	−27.31 (−51.75, −2.88)	0.028
Cough severity, VAS, mm[#]	−0.8	−11.1	−12.23 (−23.22, −1.25)	0.029
CRS, C5 (μM)[§]	+5.1	+15.1	3.12 (−13.59, 19.82)	0.720

[^], Baseline period, visit 1 (before treatment), treatment period, visits 2 and 3 (on treatment), treatment cessation period, visits 4 and 5 (off treatment); [#], mean; [§], GEM; *, baseline differences adjusted for; C5, concentration of capsaicin required to induce five coughs; LCQ, leicester cough questionnaire; Cough VAS, cough visual analogue score.

proven success of speech pathology treatment as part of the treatment programme for chronic cough, however, the mechanism behind the improvement had not been determined. It could be due to active cough suppression, reduced cough sensitivity or increased cough threshold from reduced laryngeal irritation.

In a subsequent study by Ryan *et al.* (4) objective measures such as cough reflex sensitivity testing and cough frequency were used to determine whether the treatment response was due to reduced underlying cough sensitivity or to more deliberate control exerted by individual patients. The number of treatments required to effect a response was also assessed. Following speech pathology treatment there was a significant improvement in cough related quality of life, LCQ, objective cough frequency, and cough reflex sensitivity. This study treated patients with cough that was refractory to usual medical care with or without the presence of PVCM and investigated the mechanism of action. Generally, a patient needed 3 to 4 speech pathology treatment sessions and the response was maintained after the intervention ceased. The mechanism behind the improvement was due to reduced laryngeal irritation that results in decreased cough sensitivity.

Speech pathology treatment for chronic cough is now standard practice in Australia with the Australian cough guidelines summary statement (59) being released in 2010. Most of the speech pathology treatment for chronic cough literature in the last five years has come from Vertigan *et al.* (4,5,45,49,59,61) however, other contributions come from Morrison & Schindler (60), Murry & Sapienza (3), and Gaziano & Serrano (44) from the field of otolaryngology. While Chamberlain *et al.* (43) and Pacheco *et al.* (46) investigated speech pathology and cough suppression therapy from a pulmonary point of view.

Pharmacological approaches to cough

Recent pharmacological approaches to treating chronic cough have originated from the field of otolaryngology with case reports that investigate the treatment of neuropathic cough or, laryngeal sensory neuropathy with cough as the primary symptom and their treatment with neuromodulating medications such as gabapentin (50,52), pregabalin (54) and amitriptyline (57).

Gabapentin

To investigate the effectiveness of gabapentin treatment on refractory chronic cough a double-blind randomised controlled trial comparing gabapentin to a matching placebo has been recently completed (9). Objective and subjective measures of cough were taken before, during and after treatment in adult non-smoking refractory cough participants who were randomly assigned to treatment with gabapentin 1,800 mg/day maximum tolerable dose or to a matching placebo dose over a period of ten weeks. Investigators and participants remained blinded to the treatment until completion of the study. There were no inherent differences between the two participant groups prior to treatment and when the treatment was undertaken it was found that patients on gabapentin responded positively when taking the medication unlike those patients on placebo. Gabapentin significantly improved cough specific quality of life, and reduced cough severity and cough frequency (*Table 2*). The onset of action of gabapentin was within four weeks and the effect was maintained during maximal dosing at eight weeks. However, the improvement in cough specific quality of life LCQ was not sustained after treatment withdrawal and the LCQ score returned to

baseline. A similar trend was seen in cough visual analogue score (VAS). These results indicate that gabapentin may be an effective therapy for refractory chronic cough and the reduction in efficacy of gabapentin after withdrawal further supports its antitussive effect. The treatment was generally well tolerated apart from a low frequency of expected side effects that were managed by dose reduction (9).

Peripheral cough reflex sensitivity to capsaicin did not change significantly suggesting that gabapentin did not act by reducing peripheral sensitisation. The authors draw on similarities between refractory cough and other conditions with central sensitisation (77), such as neuropathic pain. Paraesthesia (abnormal sensation in the absence of a stimulus), hyperalgesia (pain triggered by a lower level exposure to a known painful stimulus), and allodynia (pain triggered by a non-painful stimulus) are all features of neuropathic pain. These are similar to the clinical features of refractory chronic cough such as an abnormal throat sensation or "tickle" representing laryngeal paraesthesia, increased cough sensitivity in response to known tussigens (hypertussia), and cough triggered in response to nontussive stimuli such as talking or cold air (allotussia) (8). Gabapentin is effective for neuropathic pain with central sensitisation (20) and in this study, participants presenting with symptoms of central sensitisation were found to have an enhanced response to the gabapentin treatment (9).

This study supports the finding of previous case studies but improves on those by including validated objective and subjective cough measures in the comparison of gabapentin to placebo in refractory chronic cough patients. Further, it has stimulated discussion (78) and investigation (53) into considering chronic cough as a neuropathic disorder and treating it as such.

TRP antagonists

Findings suggest that the pathophysiology of sensory laryngeal hypersensitivity is related to airway mucosal TRP receptors in the sensory nerves, not only to the TRPV1 but also to the TRPA1 receptors (79). In the airways, the physiological effect of TRPV1 activation is demonstrated by the response to inhalation of capsaicin (80). There is a fivefold increase in the number of nerve profiles that express TRPV1 in airway biopsies from subjects with chronic cough compared with normal controls (10). TRPV1 and TRPA1 antagonists modify C-fibre function and reduce peripheral sensitization.

McLeod *et al.* (36) examined the molecular pharmacology

and *in vivo* effects of the TRPV1 receptor antagonist, N-(4-Tertiarybutylphenyl)-4(3-cholorphyridin-2-yl)-tetrahydro-pyrazine (2H)—carboxamide (BCTC) on the guinea pig TRPV1 cation channel. High dose BCTC (3.0 mg/kg, i.p.) produced a maximum inhibition of capsaicin-induced cough of 65%. V112220 (a pyridazinylpiperazine analog of BCTC) has also been shown to effectively decrease coughs evoked by capsaicin aerosol exposure in the guinea pig by 70% (81).

The TRPA1 antagonists GRC17536 (82) and HC-030031 (83) have shown to be effective in neuropathic pain and GRC17536 was shown in a vivo model of asthma to have an effect on airway inflammation, bronchoconstriction and cough. Pharmacology and toxicology studies confirm a good safety profile and it currently awaits clinical testing.

Whether therapeutic intervention of TRPV1 results in an improved quality of life in patients suffering from refractory cough remains to be tested in the clinic (84). Since antitussives available to control cough are often not effective (85) more potent antitussives are needed. TRPV1 antagonists may represent a potential class of antitussives that could be useful in the control of chronic persistent cough (84).

Conclusions

Implications for practice

Cough remains a major unmet clinical need in respiratory medicine (40) however, recent additions in its treatment have arisen and include behavioural approaches such as speech pathology and cough suppression therapy to pharmacological approaches such as neuromodulating medications like gabapentin. Recognising the similarities between chronic cough symptoms and chronic pain symptoms has led to a reinterpretation of cough triggers with a focus on behavioural and pharmacological treatments that can target central sensitisation processes (8,9). Speech pathology treatment for chronic cough has recently been included as standard treatment in the Australian cough guidelines and further RCT studies into this treatment will result in its inclusion in other national cough guidelines. The addition of gabapentin to cough practice guidelines should also be considered as it results in a significant improvement in cough and is well tolerated (9).

Implications for research

This review highlights the need for more randomised

controlled trials to replicate recent findings with gabapentin treatment of cough. Other neuromodulating medications such as pregabalin and their role in the treatment of cough should also be investigated. TRP antagonists need to be investigated in a clinical forum as they have shown significant promise in the laboratory. Central mechanisms are important in chronic cough and the use of magnetic imaging in clinical studies would confirm this. The concept of laryngeal hypersensitivity and its association with cough needs to be validated and measurement tools for this concept expanded on and investigated. Further RCT studies into speech pathology treatment and effectiveness of its specific components would also be useful.

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High flow on the rise—pediatric perspectives on the FLORALI trial

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Over the last decade, noninvasive positive pressure ventilation (NIV) has been increasingly used in adult and pediatric medicine to reduce the lung injuries, pneumonia, and denutrition associated with mechanical ventilation, which is implicated in the high mortality observed in these patients (1). For adults with acute exacerbations of chronic obstructive pulmonary disease (2) or severe cardiogenic pulmonary edema (3), it is now an evidence-based practice. It may also be an effective strategy in the perioperative period, provided that patients are carefully selected (4). The benefits of NIV have nevertheless not been clearly established in clinical situations like status asthmaticus (5) or acute hypoxemic respiratory failure (AHRF) or as a means to facilitate earlier extubation (6). The conflicting results for ARHF may be due to its several etiologies, which has prompted new trials with less heterogeneous patient groups (7).

FLORALI was a multicenter open-label trial performed in 23 French and Belgian ICUs from the European network of research in artificial ventilation (8). This study examined whether high-flow oxygen therapy or NIV therapy would reduce the rate of endotracheal intubation and improve outcomes in patients with AHRF in comparison with standard oxygen alone. The study had many strengths: the very high number of ICUs and the carefully predefined criteria for inclusion and exclusion ensured the selection of a homogeneous group of 525 patients with PaO_2/FiO_2 <300 and no hypercapnia or chronic respiratory failure; a respectable 60% of the eligible patients were included and randomized into one of the three groups, suggesting that the results could be generalized to other ICUs; and the rigorously designed protocol and robust data analysis ensured very reliable clinical findings. The primary outcome of the study, intubation within 28 days of randomization, did not significantly differ between groups, although the range was from 28% with high-flow oxygen to 50% with NIV. However, post-hoc subgroup analysis of patients with the most severe hypoxemia, i.e., those with PaO_2/FiO_2 <200, revealed a lower intubation rate in the high-flow oxygen group than in the other two groups. A secondary outcome of particular importance, survival in the ICU and at 90 days, was improved with high-flow oxygen, with the hazards ratio for death at 90 days being 2.1 (range, 1.01-3.99) with standard oxygen and 2.5 (range, 1.31-4.98) with NIV versus high-flow oxygen. The number of ventilator-free days between day 1 and day 28 was higher in the high-flow oxygen group than in the other two, in the overall population and in the patients with the most severe AHRF.

A recent survey in North America and Europe estimated that AHRF prevalence in pediatric intensive care units (PICUs) is 10.8% (9). The use of NIV in children with AHRF has increased over the last decade, and this respiratory assistance is now considered as the first-line treatment in 15-20% of patients (10,11). Physiological studies have demonstrated that NIV is able to unload the respiratory muscles and improve alveolar ventilation and gas exchange in children with acute moderate hypercapnic respiratory insufficiency (12). However, data supporting the usefulness of NIV remain scarce. No trial involving the collaboration of a large number of centers, as for the FLORALI study, has been conducted in pediatrics to assess the clinical management of acute lung injury. Only one bicentric randomized controlled trial (RCT) compared NIV with bilevel positive airway pressure to standard treatment, i.e., mask oxygen, in 50 young infants admitted to a PICU for AHRF (13). As in FLORALI, pneumonia was the main condition precipitating respiratory failure, and most patients

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suffered from severely impaired oxygenation with baseline mean PaO$_2$/FiO$_2$ <200 in both groups. NIV improved oxygenation, reduced respiratory effort and diminished the need for intubation by 47%. Another more recent RCT showed that bilevel NIV improved clinical scores and physiological measurements, but this study involved only children with status asthmaticus (14). In the context of moderately severe acute viral bronchiolitis, two randomized trials found short-term improvements in respiratory distress signs, blood gases and respiratory muscle load in infants receiving continuous positive airway pressure (CPAP) versus conventional oxygen therapy (15,16). Conversely, numerous retrospective or prospective pediatric observational studies have repeatedly reported the association between AHRF and NIV failure, with intubation rates of 50-80% (11,17-19) in acute respiratory distress syndrome (ARDS). Several early predictors of subsequent intubation have been identified, particularly SpO$_2$/FiO$_2$ (19), FiO$_2$ (20), pH (21), and respiratory or heart rate (17,22) evolution in the 1-6 hours following NIV initiation. A recent Pediatric Acute Lung Injury Conference Consensus stated that NIV should not be indicated in severe pediatric ARDS (23). If initiated, a close monitoring is essential to recognize the early signs of failure which indicate prompt recourse to invasive ventilation (19).

The particularly disappointing performance of NIV in the FLORALI study—a failure rate of nearly 60% in the most severe subgroup—will certainly not prompt a change in this recommendation. Two remarks can nevertheless be made about NIV management. First, in the FLORALI study NIV was used for approximately 8 hours on the first 2 days, whereas it is currently used 24 hours per day in children with moderate-severe hypoxemic failure. It can be argued that derecruitment due to intermittent NIV could be deleterious and may increase the work of breathing of the NIV group, ultimately worsening the outcome. Second, the patients experienced no improvement in respiratory comfort following NIV initiation. Discomfort is also commonly observed in the practice of NIV in children. Agitation may occur, particularly during the initial placement of the mask, but this poor tolerance rarely forces to discontinuation (24). A few studies have demonstrated the role of discomfort in NIV failure and assessed the optimal pharmacological support to provide in this context. A recent prospective, observational, multicenter study that included 390 episodes of NIV found that sedatives were used in 49.2% of cases (19). Discomfort may have multiple origins, including gastric distension, skin breakdown caused by the mask, and conjunctivitis due to air leaks (23). Another very

common source of patient's discomfort is patient-ventilator asynchrony, particularly frequent in infants and in children during NIV with pressure support (25). Three recent studies indicated that neurally adjusted ventilatory assist (NAVA) mode during NIV improved the patient-ventilator interaction (25-27). Further trials are required to assess whether NIV specific ventilators with automatic triggers and/or neural trigger of NIV NAVA are more comfortable and efficient than NIV done with conventional ventilators in pediatric AHRF.

The most striking finding from FLORALI was the consistent results in favor of oxygen delivery through high-flow nasal cannula (HFNC). This finding was particularly surprising because HFNC did not seem to provide greater respiratory support compared with NIV (28). In pediatrics, as well, there is currently strong agreement that HFNC has not been shown to be as effective as NIV (23). In addition, the pathophysiological mechanisms involved in alleviating respiratory distress have not been fully demonstrated with this device. Yet despite these considerations, HFNC use is rising in popularity because the system is easily set up and is well tolerated by patients. The heated and humidified mixture of air and oxygen is administered at a flow generally close to 2 L/kg/min in infants and 1 L/kg/min in children (29). As suggested by the self-report dyspnea and comfort scales in FLORALI, the gas mixture conditioning may rapidly improve inspiratory flow and reduce the sensation of respiratory distress. In a physiological study, we demonstrated that a nasal cannula with a flow rate equal to or above 2 L/kg/min was able to generate a CPAP ≥4 cmH$_2$O in infants with acute viral bronchiolitis (30). Although modest, this increase was clinically relevant, with an approximately 50% reduction in respiratory effort and rapid improvement in breathing pattern and respiratory distress signs. Measurements of diaphragmatic electrical activity and esophageal pressure swings confirmed the effectiveness of this device to reduce the work of breathing (31). In this disease, it is likely that HFNC mainly offsets the patient's inspiratory effort to overcome intrinsic end-expiratory pressure, thereby decreasing the dynamic collapse of the very compliant airways at this young age. The favorable effect of this technique on the ventilation/perfusion ratio, however, has not been clearly established. A theoretical explanation is that washout of nasopharyngeal dead space increases the rate of minute ventilation that participates in gas exchange (28). On a practical level, the FLORALI results suggest that HFNC is superior to the other strategies in cases of AHRF because it is able

to match patient inspiratory demands. Several RCTs with HFNC are currently underway in neonates, children and adults to examine these important issues. We coordinated a multicenter, randomized, noninferiority trial during the 2014-2015 respiratory syncytial virus (RSV) epidemic season (TRAMONTANE study, NCT02457013). One hundred and forty-two infants were assigned to treatment with either HFNC (2 L/kg/min) or nasal CPAP (7 cm of water). The primary outcome was treatment failure within 24 hours. Two other RCTs, conducted in the British Columbia Children's Hospital (Hi-Flo study, NCT01498094) and the Children's Hospitals and Clinics of Minnesota (HHFNC study, NCT01662544), compared length of hospital stay and respiratory distress score with HFNC as opposed to standard therapy for RSV bronchiolitis. These trials are now completed and should provide clinicians with evidence on HFNC efficacy for treating this leading cause of AHRF in pediatrics. Meanwhile specific research is conducted in pediatrics for severe hypoxemic patients, a cautious use of both HFNC and NIV should be recommended.

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Footnote

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High-flow oxygen cannula: a very effective method to correct severe hypoxemia

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Nasal high-flow (NHF) oxygen administration through a cannula is a simple and very effective way to correct severe hypoxemia. Initially developed in neonatal units, the system has been more recently adapted for use in adults. Several systems are now available, delivering warm, humidified oxygen at flows of up to 60 L/min. In addition to low costs and ease of application, NHF has several distinct features. First, the high flow rates can flush expired CO_2 from the upper airways and reduce dead space. Second, it creates a small continuous positive airway pressure (CPAP), typically of 2-4 cmH_2O. Third, the high flow, which exceeds the peak inspiratory flow of the patient, can limit the entrainment of room air during inspiration, and can thus provide a more stable oxygen concentration in the inspired gases. Fourth, the heated, humidified oxygen administered at high flow rates may help to prevent the stagnation of secretions and the formation of atelectasis. These features contribute to make the system particularly well-tolerated by patients with severe respiratory failure. Maggiore *et al.* (1) recently showed that use of NHF after extubation resulted in better oxygenation than with Venturi mask oxygen therapy for the same set FiO_2, and was associated with better patient comfort, fewer desaturations and interface displacements, and a lower reintubation rate.

Non-invasive ventilation (NIV) is clearly a different concept. NIV can increase end-expiratory lung volumes, decrease the work of breathing, and also improve gas exchange. On the other hand, with its interfaced equipment, NIV may increase dead space. Despite some encouraging observations (2), CPAP with or without NIV may be hazardous in the management of the acute respiratory distress syndrome (ARDS) (3). In a multicenter cohort study, Antonelli *et al.* (4) reported that the use of NIV as first-line therapy for ARDS was followed by endotracheal intubation in close to 50% of cases, especially in the most severe cases or when gas exchange did not improve after 1 h. Hence, NIV may be tried in less severe cases, especially in expert hands, but should not be used for long if there is no clear clinical response. NIV may be better used in the presence of severe hypoxemia due to cardiogenic lung edema (4,5), because of the beneficial cardiac effects of the increase in intrathoracic pressure decreasing left ventricular afterload.

In a recent multicenter study, Frat *et al.* (6) demonstrated the value of NHF in 313 patients with severe hypoxemia: 3/4 of the total population had bilateral chest infiltrates and about 2/3 had community-acquired pneumonia. Patients were randomized into three groups: treatment with NHF, with regular oxygen therapy by face mask, or with NIV (at least 8 h a day for at least 2 days). Although the differences were not statistically significant, the 28-day intubation rate (the primary outcome) was 38% in the NHF group versus 47% in the standard oxygen group and 50% in the NIV group. Interestingly, the mortality rate was significantly lower with NHF than in the two other groups, and remained so after adjustment for severity. Were these differences in mortality due only to chance? It is hard to say, as the study was limited to about 100 patients per group and was, not-surprisingly, unblinded. The mortality rates were recorded over a 90-day period, but the differences were already present after 14 days. The study clearly highlights the potential role of NHF in patients with severe hypoxemia, and supports previous observations. It also stresses the very limited place for NIV, if any, in the management of severe hypoxemia, as NIV had no benefits

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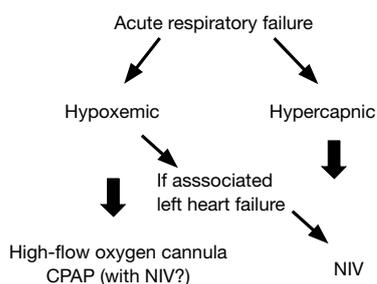
Figure 1 The different uses of high-flow nasal oxygen and non-invasive ventilation (NIV) in patients with acute respiratory failure. CPAP, continuous positive airway pressure.

over simple oxygen administration. In a slightly different setting of postoperative care, a recent study by Stéphan *et al.* (7) indicated that NHF was as effective as NIV (applied by bilevel positive airway pressure) in the prevention of respiratory failure in 830 patients after cardiothoracic surgery.

NHF is becoming the preferred option for the management of severe hypoxemic respiratory failure, and is superior to NIV in this condition. In contrast, NIV remains well established in the management of hypercapnic respiratory failure, since the landmark study by Brochard *et al.* 20 years ago showing that NIV was associated with reduced need for endotracheal intubation, length of hospital stay, and in-hospital mortality in patients with acute exacerbations of chronic obstructive pulmonary disease (8). NIV should be used cautiously in purely hypoxemic respiratory failure, unless there is associated left heart failure. The management of these different entities is, therefore, clearly different, as illustrated in *Figure 1*.

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Noninvasive respiratory support for acute respiratory failure—high flow nasal cannula oxygen or non-invasive ventilation?

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For patients with acute respiratory failure there may be advantages to the avoidance of invasive mechanical ventilation, i.e., ventilation via endotracheal intubation. Indeed, soon after the introduction of invasive mechanical ventilation many complications of positive pressure ventilation were identified (1,2). Some are directly related to the intubation procedure, such as cardiac arrest following endotracheal intubation, and laryngeal or tracheal injury leading to long-term sequelae. Others are related to the fact that the endotracheal tube adversely affects pulmonary host defenses (e.g., cough, mucociliary transport) setting the stage for ventilator-associated pneumonia, that carries its own risk of morbidity and mortality (3). Invasive mechanical ventilation generally requires sedation, which itself is often a cause of prolonged weaning and prolonged mechanical ventilation.

These major safety considerations prompted the development of non-invasive methods for delivering respiratory support without the need for intubation. Convincing evidence that non-invasive ventilation (NIV) diminishes the risk of infectious complications has been obtained from randomized controlled trials and Meta-analyses, as well as from large cohort studies and case-control studies, which have demonstrated substantial decreases in all categories of nosocomial infection (3-7). With NIV, sedation is usually not required or, if necessary, it is administered at low doses (6). By averting airway intubation, non-invasive methods of respiratory support leaves the upper airway intact, preserves airway defenses, and allows patients to eat, vocalize normally, and clear secretions more effectively.

Strengthening the rationale for the use of non-invasive respiratory support is evidence that has accumulated over the past decade that NIV lowers morbidity and mortality rates of selected patients with acute respiratory failure and may shorten hospital length of stay (8), thus reducing costs. NIV is now considered the ventilatory mode of choice in acute respiratory failure due to chronic obstructive pulmonary disease (COPD) exacerbations (9-11), acute cardiogenic pulmonary edema (12,13), and hypoxemic failure in immunocompromised patients (6,14), and for facilitating extubation in patients with COPD who fail spontaneous breathing trials (15). NIV use in these conditions is underpinned by a sound physiologic rationale—in COPD, NIV can address several of the major abnormalities in respiratory mechanics, allowing the patient to generate larger tidal volumes with less effort; in cardiogenic pulmonary edema, NIV decreases left ventricular afterload, and reduces left and right ventricular preload. By contrast, the beneficial effects of NIV remain unclear in patients with *de novo* acute hypoxemic respiratory failure, that is, non-hypercapnic patients having acute respiratory failure in the absence of a cardiac origin or underlying chronic pulmonary disease. NIV is more likely to fail in hypoxemic patients (16), and NIV failure could be associated with increased mortality (17). In unselected patients admitted to ICUs for acute hypoxemic respiratory failure, the rate of intubation is particularly high, reaching

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60% (17,18), and their in-ICU mortality after intubation may exceed 60% (17,18). Thus, NIV may improve outcome of patients who succeed in NIV by avoiding intubation, but may worsen outcome by delaying intubation in those having failed NIV.

Over the past 2 decades, systems to deliver heated and humidified oxygen at high flows through nasal cannulae have been developed as an alternative to standard oxygen delivery systems and NIV. Not withstanding the success of NIV for certain indications, high-flow nasal cannula (HFNC) oxygen delivery has been gaining attention as an alternative means of respiratory support from several clinical research groups and has been proposed as a supportive therapy in critically ill patients with acute respiratory failure (19), including post-operative respiratory failure (20), during bronchoscopy (21), or to prevent severe desaturation during intubation of patients with mild-to-moderate hypoxemia (22). The apparatus comprises an air/oxygen blender, an active heated humidifier, a single heated circuit, and a nasal cannula. At the air/oxygen blender, the inspiratory fraction of oxygen (FiO_2) is set from 0.21 to 1.0 at a flow of up to 60 L/min. The gas is heated and humidified with the active humidifier and delivered through the heated circuit.

Theoretically, HFNC has a number of advantages over other respiratory support systems, including conventional nasal cannula, face masks, or NIV. First, because gas is generally warmed to 37 ℃ and completely humidified in HFNC circuits, mucociliary function remain intact and patients report minimal discomfort (23). This is often in contrast to the delivery of low flow oxygen which is generally not humidified, leading to patient complaints such as dry nose, dry throat, and nasal pain (24,25). Insufficient heating and humidification leads to poor tolerance to oxygen therapy. Second, with HFNC the flow demands of patients are better met, maintaining the inspired FiO_2 relatively constant (26). HFNC generates a higher flow rate compared to other oxygen delivery systems, exceeding the patient's peak inspiratory flow rate in most cases. For example, during hypopharyngeal oxygraphy studies (26), during nose breathing at rest, above a flow rate of 30 L/min using HFNC the measured FiO_2 was close to the delivered FiO_2. Using conventional devices, oxygen flow is usually <15 L/min. However, the inspiratory flow of patients with respiratory failure varies widely in a range from 30 to more than 100 L/min. The difference between patient inspiratory flow and delivered flow is large, leading to entrainment of room air with the delivered gas, thus resulting in variable and lower than expected FiO_2 (27).

Third, although delivered through an open system, high flow overcomes resistance against expiratory flow and creates positive nasopharyngeal pressure (28). While the pressure is relatively low compared with closed systems, it is considered adequate to increase lung volume or recruit collapsed alveoli (29,30). A further advantage of HFNC is the wash out of carbon dioxide in anatomical dead space. Breathing frequency is lower with HFNC, while $PaCO_2$ and tidal volume remain relatively constant indicating that dead space is reduced (19,31,32). These results suggest effective carbon dioxide washout with HFNC. Finally, another major difference between NIV and HFNC is the interface. While interfaces for NIV increase anatomical dead space, those for HFNC actually decrease dead space.

Until now, only anecdotal case reports, case series and some preliminary controlled trials have provided an evidence base to guide the use of HFNC in adults with respiratory failure. The recently published FLORALI (high flow oxygen therapy for resuscitation of patients with acute lung injury) study (33), provides much needed randomized controlled trial data on the types and severities of hypoxemic respiratory failure that are most likely to benefit from HFNC. This multicenter 310 patient trial was designed to assess the rate of endotracheal intubation and other clinical outcomes among three groups: high-flow oxygen (heated and humidified air/oxygen mixture at a gas flow rate of 50 L/min applied via large-bore binasal prongs), standard oxygen therapy, and noninvasive ventilation for patients with acute, nonhypercapnic, hypoxemic respiratory failure [ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO_2:FiO_2), ≤300 mmHg]. The trial excluded patients with a history of chronic respiratory disease, including COPD, as well as patients with cardiogenic pulmonary edema, severe neutropenia and hypercapnic patients ($PaCO_2$ >45 mmHg), as NIV has already demonstrated a reduction in the intubation rate and mortality in these patients.

The primary outcome, the rate of endotracheal intubation, did not differ significantly among the groups (high flow 38% vs. standard 47% and NIV 50%) (P=0.18). However, in a post hoc adjusted analysis that included the 238 patients with severe initial hypoxemia (PaO_2:FiO_2, ≤200 mmHg), the intubation rate was significantly lower among patients who received high-flow oxygen than among patients in the other two groups (P=0.009).

In the entire cohort of 310 patients, the high-flow oxygen significantly increased the number of ventilator-free days and also reduced 90-day mortality, compared with

standard oxygen therapy (P=0.046) or NIV (P=0.006). As compared with the other strategies, high-flow oxygen was associated with less respiratory discomfort and a reduction in dyspnea, as measured by validated assessments of patient comfort. Because there was a lower respiratory rate than was observed with the other strategies at the same partial pressure of arterial carbon dioxide, it appears that the system for delivering high-flow oxygen through a nasal cannula also decreased the pulmonary dead space.

What conclusions can we draw from this study? The safety and efficacy of HFNC in non-hypercapnic respiratory failure appears to be superior to NIV or conventional facemask oxygen. However, the study does have some limitations including population itself, the use of NIV therein, the relatively small sample size, and the failure of the study to meet its primary endpoint. Just over 3/4 of the patients in each group had pneumonia, while the same proportion of patients had bilateral infiltrates on chest radiograph, thus fulfilling the criteria for acute respiratory distress syndrome (ARDS). The use of NIV in this patient population is open to question.

The pathophysiologic rationale for NIV use in pneumonia and ARDS is less sound. Unlike exacerbations of COPD, hypoxemic respiratory failure is frequently not associated with frank ventilatory failure, at least in the initial phase. NIV does not address the key pathophysiologic abnormalities of the disease, and in fact a beneficial effect on gas exchange and dyspnea may mask disease deterioration. This could lead to life threatening respiratory failure in case NIV is subsequently interrupted. Therefore, there is likely a severity window for delivering NIV as a preventive support beyond which its use may contribute to harm (34).

Robust large randomized controlled trials of NIV for acute respiratory failure (non-COPD, non-hypercapnic) are relatively scarce, and because of the heterogeneity of causes, studies fail to show that all patient subgroups with hypoxemic respiratory failure benefit equally from NIV. For example, acute pneumonia has long been considered a risk factor for NIV failure (35). A trial evaluating NIV use in heterogeneous respiratory failure showed very poor outcome in the group of patients with pneumonia, with all such patients requiring intubation (36). Another study evaluated NIV use in patients with hypoxemic respiratory failure and identified community acquired pneumonia as a subcategory with a high NIV failure rate (50% intubation rate) (35). A randomized trial showed benefit of NIV in patients with severe community acquired pneumonia, but only in the subgroup with underlying COPD (37). Other

studies (7,38), with more rigorous patient selection (such as no alteration in the state of consciousness, absence of organ dysfunction, abundant secretions, cardiac arrhythmias or ischemia) have shown some benefit in patients with acute respiratory failure (including pneumonia) treated with NIV. However, large observational studies describing the use of NIV in pneumonia have often shown high rates of failure (17,35).

Observational studies and subgroup analysis of randomized controlled trials have also identified ARDS as a strong predictor of NIV failure (35,39,40). A multicenter survey (41) evaluated NIV as first-line therapy in early ARDS patients and found that a higher severity score and a $PaO_2:FiO_2$ less than or equal to 175 mmHg 1 hour after initiation of NPPV were independently associated with NIV failure. This survey showed that, with NIV use, intubation was avoided in no more than 50% of patients, even in experienced centers. The recent Berlin definition of ARDS suggested that NIV may be indicated only in mild ARDS, and not in severe and moderate ARDS, but also emphasized that the role of NIV in ARDS has to be further evaluated (42). NIV failure in ARDS patients is highly predictable in case of shock, metabolic acidosis, high severity scores of illness, and a greater degree of hypoxemia (40).

Moreover, many patients with ARDS may not be favorable candidates for NIV due to the need to deliver lung protective ventilation. During NIV, high transpulmonary pressure swings and large tidal volumes may be generated, which could lead to the development of ventilator-induced lung injury (VILI) and contribute to the poor outcome observed in intubated patients who fail NIV. Most patients with hypoxemic ARF have a high respiratory drive, and it has been shown experimentally that the increased drive caused by a severe metabolic acidosis may cause lung injury (43). In the study by Frat *et al.*, NIV pressure support levels of 8±3 cm of water, and a PEEP of 5±1 of water resulted in a tidal volume of 9.2±3 mL/kg.

In the FLORALI study (33), it is interesting to note that there were numerically more ICU deaths in the NIV group (27 *vs.* 12 in the HFNC group and 18 in the standard oxygen group). The unadjusted hazard ratio for ICU death in the three groups was significant only in the NIV *vs.* HFNC group (HR: 2.55, 95% CI, 1.21-5.35). At 90 days, both the standard oxygen group and the NIV group had increased risk of death, but for the standard oxygen group the confidence interval almost crosses unity (HR: 2.01, 95 CI, 1.01-3.99 for standard oxygen *vs.* HFNC, HR: 2.5, 95 CI, 1.31-4.78 for NIV *vs.* HFNC). Importantly, the authors provide some information on why those patients

died. Eighteen patients died from refractory shock in the NIV group, *vs.* six in the HFNC group and twelve in the standard oxygen group. Three died from cardiac arrest in the NIV group, *vs.* one in each of the other two groups. While the authors state, and the data indicates, that there was no significant difference among the groups in terms of the time until intubation (median 27 hrs in both HFNC and NIV groups *vs.* 15hrs in standard oxygen groups) or the reasons for intubation, it is clear that NIV can mask deterioration in patients with respiratory failure, while HFNC may simply be a more effective treatment in this patient population. At the very least, this data highlights the importance of careful patient selection for NIV in acute respiratory failure resulting from pneumonia and ARDS.

In conclusion, a growing body of evidence suggests that HFNC oxygen therapy is an innovative and effective modality for the early treatment of adults with respiratory failure associated with diverse underlying diseases. However, there is no therapy that is efficient in every patient and in every type of acute respiratory failure. The study by Frat *et al.* (33) has improved our knowledge regarding the right indication for HFNC–conscious, cooperative, non-hypercapnic patients, without chronic respiratory failure. While more randomized studies are needed to confirm the clinical advantages of HFNC over other methods in specific adult populations, HFNC should be considered for the treatment of early acute respiratory failure.

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Footnote

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Acute respiratory distress syndrome: new definition, current and future therapeutic options

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Abstract: Since acute respiratory distress syndrome (ARDS) was first described in 1967 there has been large number of studies addressing its pathogenesis and therapies. Despite this intense research activity, there are very few effective therapies for ARDS other than the use of lung protection strategies. This lack of therapeutic modalities is not only related to the complex pathogenesis of this syndrome but also the insensitive and nonspecific diagnostic criteria to diagnose ARDS. This review article will summarize the key features of the new definition of ARDS, and provide a brief overview of innovative therapeutic options that are being assessed in the management of ARDS.

Keywords: Acute respiratory distress syndrome (ARDS); pathogenesis; therapeutic options

Introduction

Acute respiratory distress syndrome (ARDS) is a life threatening respiratory condition characterized by hypoxemia, and stiff lungs (1-4); without mechanical ventilation most patients would die. ARDS represents a stereotypic response to many different inciting insults and evolves through a number of different phases: alveolar capillary damage to lung resolution to a fibro-proliferative phase (3). The pulmonary epithelial and endothelial cellular damage is characterized by inflammation, apoptosis, necrosis and increased alveolar-capillary permeability, which lead to development of alveolar edema (3). Since its first description in 1967 (4), there have been a large number of studies addressing various clinical aspects of the syndrome (risk factors, epidemiology, treatment) as well as studies addressing its pathogenesis (underlying mechanisms, biomarkers, genetic predisposition). A search of PubMed using the search terms: "Acute Respiratory Distress Syndrome" yields >20,000 journal articles. However, despite this intense research activity, there are very few effective therapies for ARDS other than the use of lung protection strategies. This lack of therapeutic modalities is certainly related to the complex, pathogenesis of this syndrome with multiple signaling pathways activated depending on the type of lung injury. In addition, the lack of sensitive and specific diagnostic criteria to diagnose ARDS has hampered progress. To partially address the latter concern a recent consensus group made a number of changes to the previous American-European Consensus Conference definition of ARDS (5,6).

In the present review article, we will summarize the key features of the new definition of ARDS, which has been recently proposed from a panel of experts. In addition, we will also provide a brief overview of innovative therapeutic options that are being assessed in the management of ARDS, including gene therapy, and the administration of mesenchymal stem cells.

Table 1 ARDS Berlin definition

The Berlin definition of acute respiratory distress syndrome	
Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities — not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload.
	Need objective assessment (e.g., echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mmHg < PaO_2/FIO_2 ≤300 mmHg with PEEP or CPAP ≥5 cmH_2O[c]
Moderate	100 mmHg < PaO_2/FIO_2 ≤200 mmHg with PEEP ≥5 cmH_2O
Severe	PaO_2/FIO_2 ≤100 mmHg with PEEP ≥5 cmH_2O

Abbreviations: CPAP, continuous positive airway pressure; F_IO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure; [a]Chest radiograph or computed tomography scan; [b]If altitude is higher than 1,000 m, the correction factor should be calculated as follows: [PaO_2/FIO_2_(barometric pressure/760)]; [c]This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

Updated definition of ARDS

ARDS is a syndrome with multiple risk factors that trigger the acute onset of respiratory insufficiency. The pathogenic mechanisms vary depending on the inciting insult, but as demonstrated on autopsy findings, there are a number of common pathological pulmonary features (7), such as increased permeability as reflected by alveolar edema due to epithelial and endothelial cell damage, and neutrophil infiltration in the early phase of ARDS. Until recently, the most accepted definition of ARDS for use at the bedside or to conduct clinical trials (1,8) was the American-European Consensus Conference (AECC) definition, published in 1994 (9). ARDS was defined as: the acute onset of respiratory failure, bilateral infiltrates on chest radiograph, hypoxemia as defined by a PaO_2/FiO_2 ratio ≤200 mmHg, and no evidence of left atrial hypertension or a pulmonary capillary pressure <18 mmHg (if measured) to rule out cardiogenic edema. In addition, Acute Lung Injury (ALI), the less severe form of acute respiratory failure, was different from ARDS only for the degree of hypoxemia, in fact it was defined by a 200 < PaO_2/FiO_2 ≤300 mmHg.

Over the past 18 years of practice, the diagnostic accuracy of the ARDS definition by AECC has been questioned. In a series of 138 ARDS patients, the definition had relatively low specificity (51%) when compared with autopsy findings demonstrating diffuse alveolar damage as assessed by two independent pathologists (10). The reliability of the chest radiographic criteria of ARDS has been demonstrated to be moderate, with substantial interobserver variability (11,12). In

addition, the hypoxemia criterion (i.e. PaO_2/FiO_2 <200 mmHg) can be markedly affected by the patient's ventilator settings, especially the PEEP level used (13). Finally, the wedge pressure can be difficult to interpret and if a patient with ARDS develops a high wedge pressure that should not preclude diagnosing that patient as having ARDS. Based on these concerns, the European Society of Intensive Care Medicine with endorsement from the American Thoracic Society and the Society of Critical Care Medicine convened an international expert panel to revise the ARDS definition (14); the panel met in 2011 in Berlin, and hence the new definition was coined the Berlin definition. The goal of developing the Berlin definition was to try and improve feasibility, reliability, face and predictive validity (14). Of interest, this definition was empirically evaluated for predictive validity for mortality compared with the AECC definition, using data derived from multi and single center clinical trials (14). There are a few key modifications (oxygenation, timing of acute onset, Chest X-ray, and wedge pressure criterion) in the Berlin definition as compared with the AECC definition.

Oxygenation

In the Berlin definition, there is no use of the term Acute Lung Injury (ALI). The committee felt that this term was used inappropriately in many contexts and hence was not helpful. In the Berlin definition, ARDS was classified as mild, moderate and severe according to the value of PaO_2/FiO_2 ratio (*Table 1*). Importantly, the PaO_2/FiO_2 ratio value

Table 2 Common risk factors for ARDS	
Direct	Indirect
Pneumonia	Non-pulmonary sepsis
Aspiration of gastric contents	Major trauma
Inhalational injury	Pancreatitis
Pulmonary contusion	Severe burns
Pulmonary vasculitis	Non-cardiogenic shock
Drowning	Drug overdose
	Multiple transfusions or transfusion associated acute lung injury (TRALI)

is considered only with a CPAP or PEEP value of at least 5 cmH$_2$O.

Timing of acute onset

The timing of acute onset of respiratory failure to make diagnosis of ARDS is clearly defined in Berlin definition. It defines the exposure to a known risk factor or worsening of the respiratory symptoms within one week. It is important to identify risk factors that explain the context of acute respiratory failure arised from (*Table 2*).

Chest X-ray

The chest radiograph is characterized by bilateral opacities involving at least 3 quadrants that are not fully explained by pleural effusions, atelectasis and nodules. In the absence of known risk factors, a cardiogenic origin of edema is to be excluded by objective evaluation of cardiac function with echocardiography. Consequently, the wedge pressure measurement was abandoned because ARDS may coexist with hydrostatic edema caused by fluid overload or cardiac failure (8).

The ARDS Berlin definition was empirically evaluated to test predictive validity for mortality (14) by using a large clinical database from multicenter and single center clinical trials that included 3,670 patients. The mortality rate was 27% for mild, 32% for moderate and 45% for severe ARDS. Moreover, the number of ventilator free days declined from mild to severe ARDS, and the more severe stages of ARDS were associated with a progressive increase in lung weight as evaluated by CT scan and shunt fraction.

Current therapies

Numerous clinical studies have been conducted in patients with ARDS, but great advances in the care of the patients are still lacking and supportive therapies remain the mainstay in the ARDS management.

Protective mechanical ventilation

There is a large body of evidence from experimental and clinical studies demonstrating that mechanical ventilation, particularly in the setting of lung injury, can exacerbate functional and structural alterations in the lung (15). It is noteworthy that mechanical ventilation not only perpetuates lung injury, but also contributes to both the morbidity and mortality of ARDS (2,16,17). The concept that the limitation of end inspiratory lung stretch may reduce mortality in ARDS patients, culminated in the NIH-sponsored multicenter study of patients with ARDS (1,18). In this trial, patients randomized to receive a lower tidal volume (Vt) [4-6 mL/kg predict body weight (PBW), and maintenance of plateau pressure between 25 and 30 cmH$_2$O] had a survival benefit. Mortality was reduced from 40% in the conventional arm to 31% in the low Vt arm (CI, 2.4-15.3% difference between groups) (1). The benefit in terms of mortality and ventilation free days did not appear to be related to the value of the lung compliance at baseline or to the underlying risk factor for ARDS (19). Of note, the survival benefit was associated with a reduction of plasma IL-6 concentration, supporting the hypothesis that a lung protective strategy limits the spill over into the systemic circulation of inflammatory mediators, which in turn may induce multiple system organ failure (17).

In addition to lung over-distention, cyclic opening and closing of small airways and alveolar units (so called atelectrauma) can also lead to lung injury (20,21). Several clinical trials have been conducted in ARDS patients to examine the effects of an "open lung" approach in which the application of recruitment maneuvers and higher

levels of PEEP may limit atelectrauma. In two randomized studies, Amato and colleagues, and Villar and colleagues examined the effect of a composite strategy that minimized tidal volume, adopted lung recruitment maneuvers, and applied a level of PEEP above the closing pressure of the lung (22,23). Although the intervention arms decreased mortality, the studies were criticized due to relatively small sample sizes and relatively high mortality in the control arms. The ARDS Network performed a second large clinical trial comparing lower *vs.* higher levels of PEEP (the ALVEOLI study) (24). The trial was stopped early for futility, showing a trend to worse outcome in the higher PEEP arm, although there was an imbalance in patient characteristics at baseline favoring the control arm; the mean age of the higher PEEP arm was higher (54±17 *vs.* 49±17, P<0.05), the mean PaO_2/FiO_2 was lower (151±67 *vs.* 165±77, P<0.05), and there was a trend to higher APACHE III scores, at baseline.

Similar results were obtained in the Canadian Lung Open Ventilation (LOV) (25) clinical trial. The PEEP values were slightly higher compared to those of the previous ALVEOLI study. The conventional arm received levels of PEEP similar to the ARMA study. The study enrolled 985 patients and it failed to demonstrate any difference in mortality in the two groups (36.4% and 40.4% in the treatment and control groups respectively). The use of rescue therapies and death from refractory hypoxemia were less in the LOV-higher PEEP group. A French multi-centre randomized control trial (EXPRESS study) (26) addressed the superiority of an open lung approach in which PEEP was titrated to the highest value possible keeping Pplat <28-30 cmH_2O. In the control arm, PEEP was set between 5 and 9 cmH_2O. In both groups Vt was <6 mL/kg PBW. Patient treated according to the open lung approach had significantly more ventilator free days and organ failure free days; however, hospital, 28-day and 60-day mortality were not different between the study groups, patients. Of note, patients who now would be considered to have moderate to severe lung injury (P/F <200) tended to have lower 28-day mortality in the higher PEEP group compared to patients treated with lower PEEP.

A recent meta-analysis that incorporated trials (from 1996 to January 2010) comparing higher *vs.* lower levels of PEEP concluded that there is no difference in mortality applying lower *vs.* higher levels of PEEP in patients with mild ARDS. However, in the subgroup of patients with severe ARDS, as defined by a PaO_2/FiO_2 <200, there was be a benefit from higher levels of PEEP (27).

Non conventional therapies in severe ARDS

Historically prone positioning, high frequency oscillatory ventilation and extracorporeal membrane oxygenation have been proposed as non-conventional therapies for life-threatening refractory hypoxemia in severe ARDS patients (28). Although all these strategies have demonstrated to improve oxygenation, their impact on mortality is controversial. In fact, two recent RCT have questioned the safety of HFOV (29,30), where promising results come from a French study in which mortality was significantly lower in patients treated with extended period of prone position (28).

The prone positioning exploits gravity and re-positioning of the heart in the thorax to recruit the lung and to improve ventilation perfusion matching. Despite improving arterial oxygenation (31,32), prone position failed to show a significant improvement in mortality (32). In a subsequent study, prone ventilation was associated with a decrease in (37.8% *vs.* 46.1%) 28-day mortality in the subgroup of patients with severe hypoxemia, but given the small numbers, definitive conclusions cannot be drawn regarding the effect on mortality in this subgroup (32). However, pending results from a recent French study seem to clearly demonstrate a lower mortality in patients with severe ARDS who were treated with longer period of prone position.

In theory, high frequency oscillatory ventilation (HFOV) encapsulates the main principles of lung protection: it delivers extremely small tidal volumes around a relatively high mean airway pressure, at high respiratory frequencies (3-15 Hz), with the goal of avoiding tidal overstretch and recruitment/derecruitment (33,34). Despite the strong physiological rationale and preliminary human studies (35,36) showing improvement in oxygenation two recent large clinical trials (29,30) of HFOV in patients with moderate/severe ARDS failed to show any improvement in survival and have questioned safety of HFOV. Both trials compared HFOV to a lung protective strategy that employed low tidal volume and higher PEEP levels to fully recruit the lung. In the OSCAR study 398 patients were randomized to HFO and 397 patients to a conventional lung protective strategy. There was no difference in mortality between the two groups (HFOV 42% *vs.* conventional ventilation 41%). In the OSCILLATE study, an excess mortality was reported in the HFOV arm and the trial was stopped early after enrolling 548 patients instead of planned 1,200 patients. In-hospital mortality was 47% in the HFO group compared to 35% in the control group

(relative risk of death with HFO, 1.33; 95% confidence interval, 1.09 to 1.64; P=0.005). In addition, 11% of patients in the conventional arm crossed over to HFOV arm for refractory hypoxemia and despite this the death rates due to refractory hypoxemia were not different between groups. Possible factors that might explain this excess mortality in the HFOV arm are a greater use of sedation, neuromuscular blocker use, and longer and higher rates of vasoactive drugs. In light of these considerations, the results of these two studies preclude the routine use of this strategy in patients with ARDS (37).

In patients with severe hypoxemic and/or hypercapnic respiratory failure, extracorporeal lung support (ECLS) techniques, including extracorporeal membrane oxygenation (ECMO), have been considered to be possible rescue therapies. The aim of this strategy is to overcome severe hypoxemia and respiratory acidosis while keeping the lung completely at rest. Despite earlier negative trials (38,39), the CESAR study suggested the benefit of ECLS in patients with severe ARDS. In this RCT, 180 patients were randomized to receive veno-venous ECMO (after transfer to a specialized center) or conventional mechanical ventilation (in regional centres). The former group had a better 6 months survival than the latter one, but critics argue that the ECMO patients received a best practice treatment in specialized centers, while the control group treatment was left to the discretion of physicians in multiple non-specialized hospitals (40). Currently there is a French-led international multicenter randomized trial evaluating the impact of early veno-venous ECMO treatment in patients with ARDS, in terms of morbidity and mortality in the first 30, 60 and 90 days. The results are expected around January 2014.

ARDS therapies other than mechanical ventilation

Over the last decade, several non-ventilatory treatments have been investigated to further improve the outcome of ARDS patients. In particular, we will focus on the role of conservative fluid strategy and the putative role of neuromuscular blocking agents (8,41).

In ARDS patients, alveolar edema formation caused by increased vascular permeability may be worsened by higher hydrostatic pressure as a consequence of fluid overload. Of note, positive fluid balance, higher values of central venous and capillary wedge pressures are independent risk factors for mortality in critical ill patients. To examine whether a more fluid-conservative strategy would impact

outcomes, ARDSnet sponsored a RCT to evaluate the effects of fluid therapy strategy aimed to limit the net fluid balance in ARDS patients without shock and renal failure requiring replacement therapy (8). Mortality at 60 days was not different between the two study groups. However, patients randomized to fluid restriction had more mechanical ventilation free days and a lower ICU length of stay compared to those patients randomized to liberal fluid intake. The two study groups were different in terms of cumulative fluid balance; in particular the liberal fluid group had positive fluid balance of 7 liters in one week with 1 L of net fluid gain each day (8).

In patients with severe ARDS as defined by PaO_2/FiO_2 <150, 48 hrs administration of non depolarizing neuromuscular blocking agent (NMBA) cisatracurium has been shown to improve oxygenation, and adjusted 90-day survival, as well as decreasing duration of mechanical ventilation and barotrauma, without increasing muscle weakness (41). Moreover, NMBAs have been shown to reduce levels of both pulmonary and systemic pro-inflammatory mediators (42). However, given the potential side effects of these medications in terms of critical illness neuromyopathy (CINM), its use should be limited to severe hypoxemic patients for a brief period.

Inhaled nitric oxide for its pulmonary vasodilator effects has been proposed to treat refractory hypoxemia reestablishing an adequate ventilation perfusion matching. Both recent randomized clinical trials (43,44) and robust meta-analyses (45,46) indicate that inhaled nitric oxide improves oxygenation over a 24 hour period of treatment. However, no benefit has been demonstrated on mortality. In addition, detrimental effects on kidney function have been documented thus limiting its cautious use to patients with severe ARDS and pulmonary hypertension.

Future non-ventilatory therapeutic options

In the last decade many molecular mechanisms have been discovered which greatly increase our understanding of ARDS pathogenesis. However, none of these new advances have been translated into effective therapies to improve outcome of ARDS patients. New therapeutic opportunities may come from gene and mesenchymal stem cells therapies. In the next sections of this review we will summarize the new findings of gene and mesenchymal stem cell therapies in animal models; these approaches hold promise in the treatment of ARDS.

Gene therapy for ALI/ARDS

Epithelial damage after lung injury is characterized by apoptosis and necrosis of type I and II alveolar cells. Epithelial damage dramatically contributes to alveolar edema formation, which is associated with increased permeability; airspace infiltration by neutrophils amplifies and sustains the lung injury. After the acute exudative phase, alveolar edema clearance and proliferation and differentiation of type I into type II alveolar epithelial cells lead to resolution of lung injury. Abnormal tissue repair, depending on the severity of tissue damage, leads to extracellular matrix deposition and fibrosis.

In the acute exudative phase alveolar flooding associated with an impaired alveolar fluid clearance is the main determinant of ventilation perfusion mismatch and subsequent hypoxia in ARDS patients. This has led to extensive research to reestablish alveolar fluid clearance and keep the lung dry. The driving force for fluid reabsorption is based on the active transport of Na^+ from the alveolar space into the interstitial space. The Na^+, K^+ transporting adenosine - 5'- triphosphate (Na^+/K^+-ATPase) together with others ion transporters such as epithelial Na^+ channel (ENaC), the cystic fibrosis transmembrane conductance regulator (CFTR) create an osmotic gradient which reabsorbs fluid from the alveolar spaces.

Based on these physiological mechanisms, recent clinical trials have tested beta agonist administration as pharmacological intervention in patients with ARDS. In fact, several *in vitro* and animal studies have previously shown that beta agonist as salbutamol activate β-2 receptors on alveolar type-1 and type-2 cells, which increase intracellular cyclic adenosine monophosphate (cAMP), leading mainly to increased AFC. In 2011 the ARDS-net sponsored the ALTA study in which 282 patients with acute lung injury, as defined by PaO_2 and FiO_2 ratio of 300 or less, were randomized to receive aerosolized salbutamol (at dose of 5 mg) or placebo every 4 hours for up to 10 days (47). Unfortunately, the trial was stopped earlier because the primary end point, ventilator free days (VFDs), had crossed predefined futility boundaries. More recently, a large multicenter RCT, performed across 46 ICUs in the United Kingdom, showed that intravenous salbutamol is even hazardous for patients with early and severe ARDS (48). In fact, patients treated with salbutamol at dose of 15 µg/kg ideal bodyweight/h had higher mortality at 28 days and lower ventilator and organ failure free days. The reason of these unfavorable outcomes seems to be related to higher rates of side effects as tachycardia, arrhythmias, and lactic acidosis in the interventional arm.

Based on the negative results of these large RCTs, gene therapy approaches to restore and potentiate the Na^+ movement across the alveolar epithelial barrier could be promising strategies to overcome the problem of systemic side effects of beta 2 receptors agonists. Transfer of α2 subunit or β1 subunit of Na^+/K^+ ATPase has been demonstrated to increase the expression of Na^+/K^+ ATPase on alveolar epithelial cells and to improve alveolar fluid clearance (49,50). In a mouse model of LPS induced lung injury, plasmid transfer of genes encoding the α1 and β1 subunits of the Na^+/K^+-ATPase were delivered to the lungs of mice using transthoracic electroporation. Delivery of plasmids expressing Na^+, K^+-ATPase subunits protected the lung from subsequent injury and partially reversed existing lung injury as demonstrated by a reduction of wet-to-dry ratios, bronco-alveolar lavage protein levels and an improvement of alveolar fluid clearance, and respiratory mechanics (51). Moreover, Adir and colleagues showed that overexpression of α2 or β1 subunit of Na^+/K^+ ATPase significantly improved alveolar fluid clearance (AFC) not only in normal lungs but also in those exposed to ventilator induced lung injury (50,52). Seven days before the beginning of mechanical ventilation, rats were treated with adenovirus that expressed α2 or β1 subunit of Na-K-ATPase. This gene therapy approach prevented the 50% reduction of AFC caused by VILI (50). Beta-adrenergic agonists improve Na^+ transport mediated by Na^+/K^+ ATPase increasing the intracellular levels of cAMP. The adenovirus-induced overexpression of beta 2 adrenergic receptor gene greatly improved AFC increasing the expression of both ENaC and Na^+/K^+ ATPase (53).

A number of studies have demonstrated the role of growth factors in increasing AFC. In a mouse model of hyperoxia and oleic acid induced acute lung injury, liposome transfer of gene encoding keratinocyte growth factor attenuated lung injury likely increasing the proliferation of alveolar epithelial cells (54,55).

Lung injury in ARDS is characterized by a pro-inflammatory increase in vascular permeability and neutrophil infiltration, which sustain alveolar edema and damage to alveolar barrier. Several studies have focused on the role of gene therapy in modulating the pro-inflammatory response in the lung. Lung gene transfer encoding for IL10 has been shown to reduce the release of inflammatory cytokines in an *ex vivo* model of donor lungs before transplantation. Ten lungs of brain death patients,

who did not match the criteria for transplantation, received 12 hour of normothermic *ex vivo* lung perfusion with or without the intra-tracheal delivery of adenoviral vector encoding human interleukin-10 (AdhIL-10). The lungs treated with this gene therapy approach demonstrated better graft function with improvement in oxygenation, pulmonary vascular resistance, and an increase in anti-inflammatory cytokines release (56). Moreover, in IL-10 knock out mice, chronically infected with Pseudomonas Aeruginosa, the adeno virus transfer of gene encoding for IL-10 produced a significant anti-inflammatory effect. Treated animals showed a reduction of IL-1β, TNFα and macrophage inhibitory protein (MIP)-1α release into the airway spaces. Moreover, this gene transfer mitigated neutrophil lung infiltration (57). Similar anti-inflammatory effects have been found with the delivery of genes encoding anti-inflammatory cytokines such as interferon protein 10 (IP-10) (58), IL 12 (59) and transforming growth factor beta-1 (TGF-β1) (60).

Heme oxygenases (HO) are essential enzymes, which degrade heme into carbon monoxide (CO), biliverdin and free iron. Due to its anti-inflammatory, anti-apoptotic and, as recently described, anti-viral properties the inducible HO isoform HO-1 is an important molecule which has been used in different genetic approaches to mitigate acute lung injury (61-63). Gene transfer of HO-1 provided lung protection against hyperoxia, influenza virus pneumonia and endotoxin mediated lung injury (61-63).

Mesenchymal stem cells

Mesenchymal stem cells (MSC) are multipotent stromal cells that can differentiate into a variety of cells types including osteoblasts, chondrocytes, adipocytes, etc. These cells can be isolated not only from bone marrow but also from fat, umbilical cord blood, placental tissue, skeletal muscle, and tendons. The International Society of Cellular Therapy published the criteria to identify MSCs: (I) adherence to plastic surfaces; (II) expression of CD105, CD73, CD90, without expressing CD45, CD34, CD14, CD11b, CD79α, CD19 and human leukocyte antigen (HLA) II; and (III) the ability to differentiate into osteoblast, adipocytes, and chondroblasts *in vitro*.

MSCs have several properties that make them promising as a therapeutic approach in ARDS. MSCs differentiating into several cell types have regenerative properties and may repair damaged tissues. In addition, they can release many molecules, which contribute to immunomodulatory

and anti-inflammatory effect. Moreover, MSCs lacking the HLA II molecules may escape the immune response after allogenic or xenogenic transplantation and may be used as carriers for gene therapy.

Recent findings describe a therapeutic role of MSCs in animal models of ARDS and sepsis. MSCs may attenuate the local and systemic inflammatory response in different mouse models of sepsis, predominantly through their paracrine immune-modulatory effect, despite their limited engraftment and differentiation in alveolar epithelial cells (64). Mei and colleagues demonstrated the immune modulatory effect of MSCs in a mouse model of LPS associated acute lung injury. The systemic administration of MSCs 30 minutes after LPS injection was associated with reduction in total cell and neutrophil counts in bronco-alveolar lavage (BAL) fluid as well as in pro-inflammatory cytokines in both BAL fluid and lung parenchyma homogenate. Of interest, the authors showed the role of MSC as carriers for the vasculo-protective gene angiopoietin 1 (ANGPT1). Mice treated with MSCs transfected with ANGPT1 had complete restoration of lung vascular permeability (65). Moreover, these results were expanded in a mouse model of sepsis in which the MSC therapy not only attenuated the systemic inflammatory response and organ dysfunction, but also improved bacterial clearance and survival trough the enhancement of phagocytic activity (66). Thus, MSCs seem to be potent immunomodulators; they may interact with circulating and tissue monocytes and macrophages and reprogram them to enhance an anti-inflammatory response.

Nemeth and colleagues demonstrated that monocytes and macrophages treated with MSCs produced large amount of the anti-inflammatory cytokine IL 10; in contrast, plasma concentrations of TNFα and IL 6 were reduced. The temporal reprogramming of monocytes induced by MSCs seems to be in part related to the production of prostaglandin E_2 (PGE_2) by MSCs. PGE_2 acting on the EP2 and EP4 macrophage receptors stimulate the production of IL 10 (67).

Conclusions

ARDS still represents a deadly form of respiratory failure with long term consequences in patient survivors and indeed, their families (68,69). Supportive therapies represent the mainstay of treatment of ARDS, whereas the limitation of end end-inspiratory lung stretch has been clearly demonstrated to reduce the ARDS associated

mortality. Adoption of the new definition may be useful to better classify patients according to severity and prognosis. Lacking of effective therapies relies on the complex pathogenesis of the syndrome characterized by different overlapping signaling pathways Gene therapy and mesenchymal stem cells may be promising novel therapeutic strategies aimed at modulate key pathophysiologic mechanisms of ARDS.

Acknowledgements

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A new free-cost e-service supporting clinicians to manage their difficult-to-treat TB cases: the ERS-WHO TB consilium

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Multidrug-resistant tuberculosis (MDR-TB), defined as TB caused by Mycobacterium tuberculosis strains resistant to isoniazid and rifampicin (the core anti-TB drugs currently in use), and extensively drug resistant tuberculosis (XDR-TB), defined as MDR-TB caused by strains resistant to at least one fluoroquinolone and one injectable second-line anti-TB drug in addition to isoniazid and rifampicin, has attracted interest at different levels (1-5).

M/XDR-TB represents a serious threat for TB control and elimination. Therefore, the World Health Organization (WHO) ensures, via its Global TB Report, monitoring and evaluation of prevalence and incidence rates as well as temporal trends of drug resistant TB at both global and regional level (2,3).

According to the latest global WHO estimates, in 2013 there were an estimated 480,000 new cases of MDR-TB with 210,000 estimated deaths. This means that 3.5% of new cases and 20.5% of previously treated cases are estimated to be affected by MDR-TB at global level (2).

The prevalence of MDR-TB is particularly high in several countries belonging to the former Soviet Union (FSU), where 9-35% of new cases and ≥50% of previously treated cases harbor MDR-TB strains (2).

Although XDR-TB has presently been reported by 100 countries worldwide, representative data from continuous surveillance or special surveys regarding the proportion of MDR-TB cases affected by XDR-TB is available from 75 countries and 5 territories only. The analysis of these data indicates that the average proportion of MDR-TB cases harboring XDR-TB strains of M.

tuberculosis was 9% (95% CI, 6.5-11.5%) and that 15 out of 75 countries reported 10 or more XDR-TB cases in the most recent year for which data were available.

Again, the highest proportion of MDR-TB cases affected by XDR-TB has been observed in countries belonging to the FSU, specifically in Georgia (20%), Kazakhstan (22.7%), Latvia (21.7%), Lithuania (24.8%) and Tajikistan (Dushanbe city and Rudaki district: 21%) (2).

Recent evidence suggests that the clinical outcome of MDR-TB cases is largely unsatisfactory (2,6-10).

In the largest ever-published cohort of 9,153 MDR-TB cases from 32 observational cohorts supporting an individual data meta-analysis, the clinical outcomes were unacceptably poor (treatment success at 54%; default 23%; failure/relapse 8%; death 15%) (8). In XDR-TB cases and cases with resistance patterns beyond XDR the outcomes were even worse, with treatment success ranging from 19% to 40%, failure/relapse from 15% to 54% and death from 15% to 35% (9,10).

The challenge of providing proper treatment to M/XDR-TB patients has been identified by the international community as one of the main reason explaining poor treatment outcomes, given the high cost and the limited availability of second-line anti-TB drugs, the frequent occurrence of adverse events, the risk of acquiring further mycobacterial resistance and the presence of associated conditions (i.e., alcohol and drug abuse; poor patients' adherence) or comorbidities (i.e., HIV infection).

WHO recommends that management of MDR-TB cases be supported by a specialized team, composed by different

medical specialists, with different competencies and perspectives (including TB clinicians/pneumologists with clinical expertise both for adults and children; physicians with surgical, radiological and public health expertise; health staff with psychological background and nursing experience). The implementation of such a committee (known as "Consilium" in most of the FSU countries) is recommended to ensure a rational use of second line drugs and avoid mismanagement of MDR-TB patients. At present, several high MDR-TB burden countries in the different WHO Regions have introduced Consilium-like committees at national or sub-national level to reach consensus on the best treatment approach for their patients.

In addition to national Consilia, the Green Light Committee (GLC), a WHO-hosted committee (recently re-organized at the Regional level) provides continuous technical assistance on MDR-TB management to countries in different ways, including country visits and on-distance email or telephone coaching while ensuring MDR-TB patients are managed as per international guidelines.

However, before launching the ERS/WHO electronic Consilium in September 2012, there were no online mechanisms facilitating peer-to-peer consultation for individual patients at global level.

Available evidence suggests that the existing Consilia either have limited experience or cover only a restricted fraction of the patients in the country or territory (11). On the other hand, the majority of low TB incidence countries do not have a similar consultation committee at national level, even though expertise to manage M/XDR-TB cases in these settings is usually limited to a small group of experts and concentrated in main MDR-TB reference centres.

A recent European Respiratory Society (ERS)/European Centre for Disease Control and Prevention (ECDC) study highlighted several clinical and public health MDR-TB management shortcomings even in some of the high income and low TB incidence countries of the European Union (EU) (12). The existence of WHO guidelines and European Standards of TB Care seems apparently insufficient to guarantee appropriate treatment of M/XDR-TB, while a rapid advice from a multidisciplinary team with clinical and public health experience at national or supranational level would help ensuring proper treatment (13-15). In countries without a formal system of consultation, specialised staff from MDR-TB reference centres usually spends a significant proportion of their working time responding to phone or e-mail clinical queries from all over the country and without any formal recognition, monitoring or support

for the service offered. In addition, the recent introduction of two new second line drugs for MDR-TB (bedaquiline and delamanid) raised completely new challenges in terms of correct identification of eligible patients, choice of the optimized background regimen, management of side effects and pharmacovigilance (16,17).

The ERS has increased its commitment, technical support and funding towards TB control and elimination in Europe over time (18-23); this was done through different initiatives, including its presidential TB plan, the European forum for TB innovation and, more recently, the Rome global consultation on TB elimination which produced the global consensus document "Framework for tuberculosis elimination in low-incidence countries" (24).

To this extent and after consultation with WHO (European Office) and ECDC, and under the umbrella of the ERS Presidential initiative, the ERS-WHO electronic TB Consilium initiative has been launched during the 30th ERS Congress in Vienna, on September 6th, 2012. The WHO regional office for Europe signed a memorandum of understanding with ERS to co-ordinate and co-manages this initiative.

Aim of this article is to describe the objectives and the results of this initiative, and to advocate for its use in Asia.

The overarching goal of this initiative is to provide scientifically sound and evidence-based advices to national Consilia and individual clinicians on how to manage drug-resistant TB and other difficult-to-treat TB cases, including co-infection with HIV and paediatric cases, at global level. Through technical guidance provided to clinicians, the main contribution and outcome of the initiative will be a public health response aimed at achieving the correct management of affected patients and prevention of further drug resistance development. Its secondary objective is to ensure monitoring and evaluation of clinical practices on the ground (diagnosis, treatment and prevention). On top of supporting clinicians, the system will also allow evaluating the trends in key challenges and pitfalls which can be addressed through human resources capacity building, including M/XDR-TB management practices (e.g., correct diagnostic and treatment prescription habits), and clinician's compliance to WHO and other international, evidence-based guidelines.

The ERS/WHO TB Consilium, accessible at www.tbconsilium.org by any physician needing clinical support, ensures a free-cost, multi-lingual (English, Portuguese, Russian and Spanish at present) internet-based consultation system able to provide suggestions on clinical management

of complicated TB cases by two independent experts in less than 3 working days. The launch of the initiative has been preceded by a call for experts (including TB clinicians, paediatricians, microbiologists, but also other professionals relevant for patient management). The expert applications have then been reviewed and validated by an expert panel based on strict technical criteria. The electronic TB Consilium platform has been then presented to different countries, national TB programme managers and individual clinicians, and launched at WHO TB Strategic Technical Advisory Group (STAG) in Geneva in 2012. In addition, ERS reached chest specialists through its own publications (newsletter, European Respiratory Journal) and its website. The TB Consilium has been also included in the curriculum of the WHO Course for TB managers and consultants aimed at training on how to apply the new WHO End TB Strategy, focused on the concept of TB Elimination. The course is conducted twice a year in Cepina/Sondalo, Italy (www.publichealthcg.com) and replicated in different continents in different languages.

Recently, a first evaluation of the e-platform's utilization has been published after the TB Consilium completed its support to the first ten clinical cases (25).

The study evaluated the core variables which are necessary to describe a TB case, including the following: origin of the expert consultation's request (country generating the request), patient's country of birth, clinical questions and clinical features of the case, performance of the platform (time to load the case, response time, need for additional request for details, problems encountered, etc.), as well as client's satisfaction (evaluated through a structured questionnaire).

The study concluded that:

(I)   Requests to the TB Consilium originated from different regions of the world, confirming that there is a global need of clinical advice on MDR-TB and difficult-to treat-cases. Requests came mainly from Europe-with the United Kingdom being predominant (5 requests out of 10) but also from India, Latin America and Vietnam;

(II)   Although the majority of requests were for adult patients (mean age, 27.9 years; range, 12-40 years), 2 out of 10 cases were of paediatric interest (a 14-year-old girl from Vietnam and a 12-year-old girl from India, respectively);

(III)   Only one among the first ten cases evaluated was drug susceptible while the remaining nine cases were MDR-TB (2 being XDR-TB and 1 pre-XDR, e.g., MDR-TB with additional resistance to a fluoroquinolone or aminoglycoside);

(IV)   Six out of ten cases were pulmonary TB cases, while three were extrapulmonary and one had both pulmonary and extrapulmonary disease. All of them were bacteriologically confirmed;

(V)   In most of the cases the clinician requested experts' opinion about the most suitable treatment regimen, validation of the treatment regimen prescribed and suggested treatment duration. In a very complicated case from the United Kingdom the clinician requested experts' opinion for the management of side effects as cachexia and muscle wasting and the appropriateness of surgical treatment. In other cases, specific guidance on possible drug interactions was requested. In one of the extrapulmonary cases the question specifically asked how to monitor the response to treatment and the recommended treatment duration;

(VI)   The time needed for the clinician to upload a single case and questions into the electronic platform was estimated to be less than 30 min, with a mean value of 20 min; the average response time was 40 h. In two cases the experts asked the client clinician to provide further clinical details in order to finalise their report properly;

(VII)   All clinicians reported to be largely satisfied by the TB Consilium services;

(VIII) Experts involved provided very useful comments to improve the platform's functionality. The main suggestion was aimed at improving reporting of the new diagnostics tests and radiological findings, and a better location for the (open) spaces where the clinician is allowed to add descriptive clinical details;

(IX)   The coordinators identified the need to allow a consolidation of the two experts' reports, in order to provide the clinician with a perspective for the best interpretation and use of the experts' reports. In addition the client's satisfaction survey was added, together with the option to invite a third expert or to disengage an expert not able to provide a rapid reply if necessary. The reviewers' pop-up directory was improved, making details available to facilitate the choice (e.g., language spoken, specialty: paediatrician, surgeon, HIV/infectious disease expert, etc.).

Further update about utilization of the Consilium platform is summarized in *Table 1*.

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Table 1 Summary evaluation report of the first 91 cases that benefited from expert advice of the ERS/WHO TB Consilium as of June 30th 2015

Indicator	N
Country requesting proportion (top 3) (%)	
India	30
Italy	18
United Kingdom	12
Country of birth proportion (main) (%)	
India	49.2
Sex (%)	
Females	43.2
Males	56.8
Age (year)	
Mean	33
Range	1-68
Paediatric cases (%)	
14 active TB	20.8
13 Latent TB Infection	19.4
Susceptibility of the M. tuberculosis strain (%)	
Susceptible	16.4
Poly-resistant	4.4
Mono-resistant	7.4
M/XDR-TB	64.1
Not classifiable	7.4
Pulmonary (P) and extrapulmonary (EP) TB (%)	
Pulmonary TB	64.1
Extra-Pulmonary TB	14.9
P/EP TB	19.4
Not classifiable	1.4
Core clinical questions (%)	
Treatment regimen	83.5
Treatment duration	6
Other	10.5
Mean time to case-load (min)	20
Average response time (h)	48
Repeated requests on the same case [n (%)]	6 (8.9)
Clinician satisfaction [n (%)]	91 (100.0)

ERS, European Respiratory Society; WHO, World Health Organization; TB, tuberculosis; XDR-TB, extensively drug resistant tuberculosis.

As of June 30th, 2015, 91 cases benefited from the ERS/WHO Consilium. There were 67 cases of active TB requesting individual advice. In addition, advice was requested for additional 24 cases, including 4 active TB and 20 individuals latently infected in two epidemics (26,27). For 6 individual cases more than one opinion was requested to the experts, in order to ensure adequate treatment monitoring at different stages of the patients' roadmap towards cure (*Table 1*).

India, United Kingdom and Italy are the top three countries requesting the ERS/WHO TB Consilium services, the majority of patients (49.2%) being born in India. The patients were young (mean age, 33 years), with a significant proportion of paediatric cases (40.2%), extrapulmonary TB forms (14.9%) and M/XDR-TB cases (64.1%). Females account for 43% of the cases.

The majority of the clinical queries are, again, focused on the most appropriate treatment regimen and its duration in M/XDR-TB cases, TB/HIV co-infected individuals and other difficult-to-treat cases which included extrapulmonary and paediatric TB cases. In 13 (19.4%) cases, the clinician requested experts' opinion about the appropriateness of introducing one of the new TB drugs to treat MDR-TB cases. In 12 cases this request was considered appropriate while in 1 case the experts suggested alternative regimens sparing the new TB drug.

The time necessary to load the cases into the platform remained stable at 20 min, while the average time necessary to provide the clinical feedback by two top global TB experts has slightly increased with the increasing number of consultations, being now at 48 h. This time include also week-ends, holiday time and non-working days, as several requests are submitted on Fridays. Clients confirmed their appreciation for the feedback received.

There are plans to add the translation of the platform into French. In addition, ERS is working hardly to further expand the platform functionality in different areas, as follows:

(I) A new function related to trans-border migrants has recently completed pre-testing, has been then shown to TB national programme managers and will be launched late in 2015 in Europe in collaboration with WHO, to be then expanded globally. It is aimed at supporting clinicians treating trans-border migrants affected by TB to liaise with the country where the patient is planning to migrate during TB treatment, so that data can be exchanged and continuum of care can be ensured.

This functionality will contribute to prevent management errors due to lack of information on the previous patient's medical history as well as early treatment interruptions after the patient's migration to a different country or destination.

(II) A new functionality is under development in collaboration with the civil society organizations, aimed at allowing patients to directly request specific advices for their TB infection or disease to a physician, a nurse, a psychologist, and/or to ask motivational support by other patients who survived the disease.

(III) Last but not least, a new functionality is under preparation to support clinicians and public health specialists in managing index cases' contacts and latently infected individuals (including those generated by outbreaks and micro-epidemics).

A plan has been developed to increase the number of experts serving the ERS/WHO TB Consilium in its different functionalities, based on the increasing workload expected in the coming months due to its expanded use in Asia and Latin America. To cope with the new challenges, new experts speaking Spanish, Portuguese and Russian have been recently added to the initial pool of TB Consilium consultants. In addition a coordinator has been identified to run the patients' branch, in collaboration with European Lung Foundation (ELF), the patients' voice supported by ERS.

At present two co-directors manage the ERS/WHO Consilium, with the support of one area coordinator. The number of area coordinators will be increased over time, to ensure timely response to all the queries posed.

All the TB Consilium functionalities have been developed based on the requirements of the Swiss legislation in force, which ensures adequate confidentiality and data protection.

The ERS/WHO TB Consilium initiative will represents an effective instrument able to support the new WHO End-TB Strategy focused on the concept of TB pre-elimination (defined as <10 TB cases per million population) and TB elimination (defined as <1 TB case per million) (23).

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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The medical and surgical treatment of drug-resistant tuberculosis

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Abstract: Multi drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) are burgeoning global problems with high mortality which threaten to destabilise TB control programs in several parts of the world. Of alarming concern is the emergence, in large numbers, of patients with resistance beyond XDR-TB (totally drug-resistant TB; TDR-TB or extremely drug resistant TB; XXDR-TB). Given the burgeoning global phenomenon of MDR-TB, XDR-TB and TDR-TB, and increasing international migration and travel, healthcare workers, researchers, and policy makers in TB endemic and non-endemic countries should familiarise themselves with issues relevant to the management of these patients. Given the lack of novel TB drugs and limited access to existing drugs such as linezolid and bedaquiline in TB endemic countries, significant numbers of therapeutic failures are emerging from the ranks of those with XDR-TB. Given the lack of appropriate facilities in resource-limited settings, such patients are being discharged back into the community where there is likely ongoing disease spread. In the absence of effective drug regimens, in appropriate patients, surgery is a critical part of management. Here we review the diagnosis, medical and surgical management of MDR-TB and XDR-TB.

Keywords: Extensively drug-resistant tuberculosis (XDR-TB); surgery; drug resistance

Introduction

The burgeoning drug-resistant tuberculosis (DR-TB) epidemic is a public health problem of global importance. Although TB incidence and mortality has decreased in several parts of the world, the overall prevalence of multidrug-resistant tuberculosis (MDR-TB) is increasing in many high-burden countries, particularly in Africa (1). According to the latest WHO statistics, approximately half a million new cases of MDR-TB are diagnosed every year (2). Of these, it is estimated that approximately 40,000 have extensively drug-resistant tuberculosis (XDR-TB). Despite this, limited laboratory capacity and lack of widespread drug susceptibility testing in resource-poor settings means that less than 6% of cases are thought to have been correctly diagnosed (2). In 2011, only one in five of the estimated DR-TB cases among patients notified in the world were enrolled on treatment (3). A large reservoir of patients with undiagnosed DR-TB thus exists that continues to drives person-to-person transmission, and threatens to destabilise global TB control (4,5).

The treatment of patients with DR-TB is complex, and characterised by a longer duration of treatment, the use of less potent but more toxic medications, higher relapse rates, and a lower likelihood of treatment success when compared to drug-susceptible TB (6). Treatment for DR-TB treatment is also considerably more expensive: a recent study by Pooran *et al.* estimated that despite only comprising 2.2% of the case burden of TB in South Africa, DR-TB consumed 44% of the total national costs of diagnosing and managing all forms of TB (~$158 million) in 2011 (7).

In this review, we outline the diagnosis, medical management and treatment outcomes, and indications

and outcomes of adjuvant resectional surgery in the management of DR-TB.

Definitions

MDR-TB is defined as resistance to at least isoniazid and rifampicin, the two most effective first-line antituberculous drugs, while XDR-TB is defined as MDR-TB plus resistance to any fluoroquinolone and any second-line injectable (either kanamycin, amikacin or capreomycin) (3). Pre-XDR-TB refers to MDR-TB resistant to either a second-line injectable drug or a fluoroquinolone.

Other terms such as extremely drug-resistant TB (XXDR-TB) (8) or totally drug-resistant TB (TDR-TB) (9,10) have been used by various authors to described strains with more extensive patterns of resistance (to all first-line and second-line drugs). These reports have given rise to the spectre of so-called "untreatable" TB, which has been sensationalized in the media. However, due to problems with the reliability and reproducibility of in vitro drug susceptibility testing for second-line drugs, no international consensus has been reached about the definition of more extensive resistance patterns, and the term "resistance beyond XDR" is preferred. The relevance of these resistance patterns on outcomes is also an active area of study.

Diagnosis of drug-resistant tuberculosis

Culture-based tests for DR-TB

For many years, the laboratory diagnosis of DR-TB has depended on the demonstration of the presence of M.tb growth in the presence of specific antituberculous drugs— so-called conventional drug-susceptibility testing (DST). Solid agar methods are the diagnostic gold standard (11), while liquid culture methods such as the Bactec MGIT 960 system (Becton-Dickinson, Sparks, MD, USA) have equivalent performance, and are WHO-endorsed (12). However, a lack of laboratory infrastructure in developing countries means that very few countries have access to any DST at all: the WHO reports that globally less than 4% of bacteriologically-positive cases and only 6% of retreatment cases were tested for DR-TB in 2011 (13). Another major disadvantage of these culture-based methods is the long delay (usually several weeks) in obtaining DST results. During these delays, regimens may be used which are not only ineffective, but which encourage the development of further drug resistance,

and crucially, allow for resistant disease to be spread. A strategy that aims to control DR-TB must therefore aim not only to increase access to DST, but also to reduce the lead-time for accurate diagnosis (14). New advances in rapid growth- and microscopy-based DST, such as the microscopy observed drug susceptibility (MODS) method and thin layer agar (TLA) technique have shortened the delay to less than two weeks, but are limited by the need for labour-intensive laboratory infrastructure (15). More recently, the direct nitrate reductase assay (NRA), a rapid, low-cost, phenotypic method based on the metabolic activity of M.tb which is usually performed on solid media, has been shown to accurately diagnose DR-TB after ~21 days when performed directly on smear-positive specimens (16).

Molecular DST

New nucleic acid amplification tests (NAATs) promise to reduce the interval between sample acquisition and susceptibility result from weeks to hours, and are also becoming increasingly automated and easy to perform. They have the potential to transform the drug-sensitive and drug resistant TB epidemic in high burden countries by providing rapid DST results at the time of TB diagnosis, increasing the number of cases that are diagnosed with DR-TB and started immediately on the correct treatment, and impacting on transmission rates (17).

Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) is a semi-nested quantitative real-time polymerase chain reaction (PCR) assay that can deliver simultaneous diagnosis of TB and rifampicin resistance in less than two hours. It is an automated, cartridge-based system that can be performed in decentralized locations, outside of reference laboratories and potentially at point-of-care, by staff with minimal laboratory training. It has been widely validated on sputum samples, although reports are also emerging on its accuracy in other respiratory specimens and extrapulmonary samples (18-21). A recent meta-analysis has reported the sensitivity and specificity of the assay for the detection of rifampicin resistance in sputum to be 94.1% and 97.0%, respectively (22). Based on this evidence, the WHO has strongly recommended that Xpert® MTB/RIF, where available, should be the first investigation in all patients suspected of having DR-TB or HIV-associated TB (23). A disadvantage is that Xpert® MTB/RIF does not assay for isoniazid resistance and therefore isoniazid mono-resistance, which has a frequency of about 10-15% in high burden settings, will be missed (24,25).

Table 1 First- and second-line drugs based on the World Health Organization classification (32)

Group 1: first-line oral TB drugs
Isoniazid (H)
Pyrazinamide (Z) or PZA
Ethambutol (E) or (EMB)
Rifampicin/rifampin (R) or (RIF)
Rifabutin (RFB)
Group 2: second-line injectable TB drugs
Kanamycin (KAN)
Amikacin (AMK)
Capreomycin (CAP)
Streptomycin (STR)
Group 3: fluoroquinolones
Levofloxacin (LFX)
Moxifloxacin (MFX)
Ofloxacin (OFX)
Gatifloxacin (GFX)
Group 4: oral bacteriostatic second-line TB drugs
Para-aminosalicylic acid (PAS)
Cycloserine (DCS)
Terizidone (TRD)
Ethionamide (ETH)
Prothionamide (PTO)
Group 5: TB drugs with unclear efficacy or unclear role in treating drug resistant-TB
Clofazimine (CFZ)
Linezolid (LZD)
Amoxicillin/clavulanate (AMX/CLV)
Thiacetazone (THZ)
Clarithromycin (CLR)
Imipenem/cilastatin (IPM/CLN)
High-dose isoniazid (high-dose H)

Another concern with Xpert® MTB/RIF is suboptimal positive predictive value in settings where the prevalence of drug-resistant TB is less than 20%. In relatively high-burden settings, even in South Africa where MDR-TB prevalence rates are ~5% to 6% (14), the positive predictive value is only likely to be approximately 70% to 80%, though the precise figure remains unclear. This means that approximately one in three or four rifampicin-resistant results will possibly be falsely positive, creating uncertainty around the decision to start MDR-TB treatment. In South Africa, the policy is to initiate MDR-TB treatment on an Xpert® MTB/RIF showing rifampicin

resistance, particularly if the patient is unwell, until further confirmatory test results on two samples (either phenotypic DST or alternative PCR-based test like Hain MTBDR*plus*) become available.

A line probe assay is laboratory-based type of nucleic acid amplification assay in which products are hybridized onto a nitro-cellulose strip. An example is the MTBDRplus assay (Hain Lifesciences), which offers similar performance to Xpert MTB/RIF for TB detection, and has excellent performance for the detection of MDR-TB (26,27). It has the advantage of interrogating for both rifampicin and isoniazid resistance. More recently, the MTBDRsl assay (second line) assay has been introduced which tests for drug-resistance to second line injectable drugs (mutations on the rrs gene), fluoroquinolones and ethambutol (28). However, this assay has diminished accuracy in smear-negative specimens (29), meaning that culture isolates still need to be awaited to rule-in resistance.

The diagnosis of extrapulmonary DR-TB is even more challenging as obtaining samples for diagnosis often requires specialized skills (e.g., lumbar puncture or biopsy), and the traditional methods of smear microscopy and culture perform poorly on paucibacillary non-sputum samples. Performing an Xpert MTB/RIF on concentrated urine can identify 40% of HIV-TB cases who are sputum-scarce (30), and sensitivity is approximately 23%, 53%, and 78% in the pleural, pericardial and CSF compartments, respectively [R. Meldau and K. Dheda, submitted; S. Pandie and K. Dheda, submitted; (31)]. In the CSF and urine compartments, centrifuging the fluid significantly improved accuracy. In BAL fluid obtained by bronchoscopy, Xpert yield was ~75% and was unaffected by HIV status (18).

Medical management of DR-TB

MDR-TB treatment

The treatment regimen for MDR-TB consists of a backbone of a later generation fluoroquinolone (moxifloxacin, gatifloxacin or levofloxacin) and an injectable aminoglycoside (either amikacin or kanamycin), any first line drug to which the isolate is susceptible, and the addition of group 4 drugs such as cycloserine/terizidone, and ethionamide, such that as least four drugs to which the isolate is likely to be susceptible are being used (see *Tables 1,2*). The intensive phase (with injectable) is eight months, followed by a continuation phase of 12 to 18 months. The recommended duration of treatment is guided by culture conversion and is usually

Table 2 Principles of management of drug-resistant TB

Principles of medical management of MDR-TB

- A regimen is based, when possible, on proven or likely susceptibility to at least four drugs
- A regimen is generally based on a backbone of a later generation fluoroquinolone (moxifloxacin or levofloxacin), and injectable agent (usually an aminoglycoside, i.e., either amikacin or kanamycin), any first line drug to which the isolate is susceptible (see *Table 1*), and addition of group 4 drugs such as cycloserine/terizidone and ethionamide, and others, such that at least four drugs to which the isolate is likely to be susceptible are being used
- The injectable drugs are used for 6-8 months, and longer in certain cases, and the total duration of treatment is suggested to be 24 months
- If the patient has previously been on treatment with a specific drug for three or more months, then this drug is generally avoided
- Addressing psychological factors to ensure compliance is critical
- Patients should be monitored for adverse drug reactions, which are common
- A single drug should not be added to a failing regimen

Principles of medical management of XDR-TB

- Regimens should be constructed based on prevailing DST patterns
- Given the high background rates of TB and MDR-TB in several countries, and lack of availability of newer agents like linezolid and bedaquiline, regimens are often constructed around a backbone of capreomycin and PAS
- Any drug that the isolate is susceptible to from category 1, and any remaining available drugs from category 3 or 4, are added to the regimen
- Patients should be carefully monitored for adverse drug reactions, particularly capreomycin (renal failure, hypokalemia and hypomagnesaemia), which are common
- Patients on capreomycin should have weekly urea and electrolytes monitored for the first eight weeks and then monthly thereafter. Attention should be paid to correcting risk factors for renal failure (dehydration, nausea, vomiting and diarrhea, avoidance of other nephrotoxic drugs (cotrimoxazole and nevirapine), and early identification of underlying renal disease (diabetes and HIV-associated nephropathy)

determined by adding 18 months to the date of the first of consecutive negative cultures; the WHO recommends a total treatment duration of at least 20 months (33). Non-adherence, incorrect drug dosage, hetero-resistance, and malabsorption should be considered in patients who do not show a clinical response and remain persistently culture-positive despite exhibiting consistent susceptibility to second-line drugs. These patients may be considered for the addition of alternative second line agents to their regimens, and/or referred for surgery after appropriate investigations are undertaken.

XDR-TB treatment

With the loss of two of the most potent groups of second-line drugs (namely fluoroquinolones and aminoglycosides), the design of a treatment regimen for XDR-TB is more complex (see *Table 2*). Extended regimens in TB-endemic countries, given the lack of availability of linezolid,

often consist of a backbone of capreomycin and para-aminosalicylic acid (PAS), with other first-line, second-line or third-line anti-TB drugs added to which susceptibility has been demonstrated, or at the discretion of the attending clinician. The intensive phase with capreomycin should be at least eight months (15). The exact number of drugs used to treat XDR-TB is not known, but most patients will receive five to six drugs. Unfortunately, high rates of capreomycin resistance (~80%) in XDR patients have been observed (E. Pietersen and K. Dheda, unpublished work), presumably due to cross-resistance with the other aminoglycosides (34); similarly, as the majority of patients with XDR-TB have been previously treated for MDR-TB (35,36), prior exposure to drugs like ethionamide and terizidone usually excludes their use. Despite documented fluoroquinolone resistance, moxifloxacin is usually added to the regimen because it has increased antituberculous activity compared with ofloxacin, and because there is differential strain-specific susceptibility to the fluoroquinolones (37).

Table 3 Criteria for selection for candidates for surgery in DR-TB
• Persistently positive smear or culture despite optimal antituberculous therapy
• Extensive isolate drug resistance pattern with high probability of failure or relapse
• Radiographically localized disease with high probability of near-total resection
• Expectation of adequate cardiopulmonary reserve post-surgery
• Presence of sufficient drug activity to facilitate healing of the bronchial stump

Moxifloxacin has been shown to be effective against isolates phenotypically resistant to ofloxacin or ciprofloxacin (38), and may be associated with improved outcomes for patients with XDR-TB (39). In isolates where lack of isoniazid susceptibility results from mutations in the promoter region of the *inhA* gene (40-42), low-level resistance can likely be overcome by increased doses of the isoniazid ("high-dose INH") (43). This pattern of resistance is often accompanied with cross-resistance to other second line anti-tuberculosis agents, specifically ethionamide, as it has a structural similarity to isoniazid (44). Other drugs like clofazamine (45) and beta-lactam antibiotics like meropenem and co-amoxiclav (46) from group 5 are also used, although good quality efficacy data is lacking. Bedaquiline, the first novel antituberculous drug to emerge in almost half a century (47), has been cautiously approved in an interim recommendation by the WHO for patients in whom a regimen containing four effective second-line drugs cannot be constructed, or in patients where there is MDR-TB plus documented resistance to a fluoroquinolone (pre-XDR-TB), provided that bedaquiline can be protected by at least three effective drugs (48). The latter is often not possible in TB-endemic countries with currently available drugs. Safety concerns have been raised about the interaction between bedaquiline and clofazamine and the fluoroquinolones, as all cause QT prolongation. The addition of linezolid to the regimen of patients failing standard XDR-TB treatment has been shown to improve culture-conversion, but longer-term outcomes are unknown, and cost and toxicity are major concerns (49). Neither bedaquiline nor linezolid are currently available to National Treatment Programs in countries where XDR-TB is prevalent and endemic.

Monitoring during treatment

Treatment for DR-TB involves the use of toxic medications: drug-associated adverse effects are common, and can frequently interrupt treatment (50). In addition to monitoring sputum cultures, it is essential to monitor renal function and potassium at least monthly during the intensive phase of treatment involving an injectable. Ototoxic hearing loss is common in patients with DR-TB treated with aminoglycosides: a recent study from South Africa found that 57% of patients had developed high-frequency hearing loss after three months of aminoglycoside treatment (51). All patients should therefore be screened monthly with audiometry during the intensive phase of treatment. Thyroid function should be monitored between six and nine months of treatment with ethionamide, prothionamide or PAS, and a full blood count should be checked monthly in patients taking linezolid.

Treatment outcomes in DR-TB

Globally, survival and treatment outcomes of drug-resistant TB vary widely depending on geographical location, regimen choice, duration of treatment, and background prevalence of TB and HIV (6), but in general, correlate with the degree of drug resistance. The overall treatment outcomes are far from satisfactory: the WHO reports that of the estimated half a million MDR-TB patients started globally on treatment in 2009, only 48% were treated successfully (2). Treatment outcomes in XDR-TB are even worse; while the overall success rate for XDR-TB in a recent meta-analysis was reported to be 44% (39), additional resistance to second- and third-line TB medications beyond the minimum definition of XDR-TB was associated with further reductions in the likelihood of success. The cure rate in high-burden countries may be even lower: in South Africa, less than 20% of patients with XDR-TB culture-converted within six months of initiation of treatment, and this poor outcome was independent of HIV status (35). There is thus a desperate need for new drugs and additional interventions to improve these outcomes, particularly in XDR-TB. A number of novel drugs are undergoing clinical testing, but are unlikely to be available for several years yet (52).

Table 4 Pre-operative workup of patients for surgery for DR-TB

- HRCT to assess for bilateral disease and to plan surgical approach (PET/CT may be a useful adjunct to confirm contralateral metabolically active nodal or parenchymal disease in borderline cases or those with radiological changes secondary to previous TB)
- Spirometry
- ECG (± echocardiogram) to exclude pulmonary hypertension
- Six-minute walking test (6MWT), quantitative ventilation: perfusion scanning and arterial blood gas measurement in borderline cases
- CD4 count in patients with HIV co-infection
- Nutritional assessment by dietician with supplementation if required

Adjuvant surgical management of

Rationale and indications for surgery

Thick walled cavitatory lesions and areas of destroyed lung contain up to 10^7 to 10^9 *M.tb* organisms (53), harbouring actively replicating bacilli even in patients who are sputum culture-negative (54,55). These tuberculous lesions have reduced exposure to host defenses, and are penetrated poorly by antituberculous drugs (56). Cavities act not only as huge reservoirs of *M.tb* infection (with the potential for intrapulmonary or contralateral spread), but also as the likely site of the development of drug resistance (57). The rationale behind surgery for DR-TB is that excision of these cavities (along with "debulking" of any necrotic or non-viable lung tissue) will dramatically reduce the overall organism burden in the lung while simultaneously removing the sites of high concentrations of drug resistant bacilli. The surgical removal of cavities is hoped to enhance the sterilizing properties of post-surgical chemotherapy and increasing the likelihood of treatment success (58,59). Complications of TB including massive haemoptysis, aspergilloma, bronchiectasis, pneumothorax, bronchopleural fistula, tracheal or bronchial stenosis and empyema remain valid indications for surgery in both drug-sensitive and drug-resistant TB (60), but these topics are beyond the scope of this review.

The indications for surgery for DR-TB have remained largely unchanged since they were first described by Iseman *et al.* in 1990 (61), (an adaptation of which is shown in *Table 3*). Potential surgical candidates include those patients with localized disease and adequate pulmonary reserve who have either: persistently positive sputum smears and/or cultures despite an adequate trial of appropriate chemotherapy; or those who have relapsed, or are thought to be at high risk of relapse based on results of drug resistance profiling or radiological findings. The lack of effective sterilizing

chemotherapy for XDR-TB means even "cured" patients remain at high risk for relapse, and may be considered candidates for resection regardless of sputum culture status. The prerequisite of the presence of sufficient susceptible drug activity to facilitate healing of the bronchial stump is also less relevant in the setting of XDR-TB, where extended resistance patterns mean that surgery often remains the only option for cure.

Timing of surgery

Ideally, surgery should be performed once culture-conversion has been achieved to minimize the risk of post-surgical complications. Performing surgery later in the course of treatment may also allow for time for nutritional supplementation and control of coexisting medical conditions. However, particularly in the case of XDR-TB (as outlined above), this is unlikely to ever be achieved. Delaying surgery and persisting with ineffective chemotherapy may only facilitate progression of disease, and further promote the development of drug resistance (62). The timing of adjuvant surgery must consider the likelihood of potential culture-conversion based on the resistance profile of the *M.tb* isolate; however, a minimum of three to six months of pre-operative chemotherapy is usually given (62-66).

An additional consideration in the setting of HIV/DR-TB co-infection is that surgery may need to be postponed until immunity has been restored with antiretroviral therapy (ART) to the point at which it is expected that major surgery can be withstood.

Preoperative workup, surgical approach and complications of surgery

The preoperative workup is directed at assessing disease extent

and estimating cardiopulmonary reserve (see *Table 4*). A high-resolution CT chest is a prerequisite to assess the presence of contralateral disease, and to plan the surgical approach. Spirometry is required to estimate pulmonary reserve, while a 6-minute walk test (6MWT) is a good test of functional capacity. In borderline cases, quantitative perfusion lung scanning can assist in estimating the degree of functional loss following surgery. Cut-off values for predicted postoperative lung function are not defined in this group of patients, but can be adapted from studies of resectional surgery for lung cancer: predicted post-operative forced expiratory volume in one second (FEV_1) should likely be greater than ~800 mL for pneumonectomy candidates (67). An ECG followed by an echocardiogram, where indicated, may be useful in excluding pulmonary hypertension, which would otherwise contraindicate surgery. Positron emission tomography-computed tomography (PET/CT) has been proposed as a noninvasive imaging method that may give additional information about tuberculous disease status, particularly about the presence of contralateral parenchymal as well as nodal metabolic activity (68,69). Its role in guiding surgery remains unclear. The preoperative improvement of nutritional status has been advocated by many authors (64,65,70,71) to improve wound healing and post-operative recovery.

The extent of anatomical resection (wedge resection versus segmentectomy, lobectomy or pneumonectomy) is determined by the distribution of radiological disease, and is balanced by the desire to remove as much pathological lung as possible while preserving post-operative pulmonary reserve. While the procedures performed in case series and cohort studies were predominantly lobectomies or pneumonectomies for unilateral disease, sequential resection of bilateral cavities in patients with adequate pulmonary reserve has also been described (70,72,73).

The surgical approach is almost always via a muscle-sparing posterolateral thoracotomy; the median approach has also been studied, but offers limited exposure for left-sided resections (74). Video-assisted thoracoscopic surgery (VATS) is associated with less wound pain, fewer pulmonary complications, and a shorter hospital stay than with a thoracotomy. This technique has recently been shown to be a feasible option for smaller wedge resections and isolated lobectomies in carefully selected patients with less extensive disease (75). The obliteration of the pleural space, the presence of infected lymph nodes in the peribronchial area, and extensive adhesions (between the lung, vasculature and the chest wall) occurring as a result of chronic tuberculous

sepsis, typically limit thoracoscopic intervention and are a contraindication to VATS (63).

Bronchial stump closure is usually by stapling, although interrupted sutures with absorbable or non-absorbable sutures (either on their own or as additional reinforcement), are also used (59,65). Muscle-flap buttressing of the bronchial stump has been advocated to prevent the post-operative complication of bronchopleural fistula especially in patients with positive sputum at the time of operation (65); however, this practice has not been universally adopted, and a series of 106 patients from South Africa in which bronchial stump reinforcement was only performed in two cases reported no cases of bronchopleural fistula formation (64).

With careful patient selection, the operative mortality in lung resection is less than 5% (62-66,76). Common complications range in frequency between 12% and 30%, and include bleeding, empyema, wound complications and bronchopleural fistula.

Outcomes of surgical treatment

There is a dearth of good quality of data supporting the use of adjuvant surgical treatment for DR-TB, and current recommendations are based on expert opinion. No randomized controlled trials have been performed, and it is likely that the available data from case series and cohort studies is biased towards surgery in patients with less extensive disease. Nevertheless, a recent systematic review and meta-analysis of 24 comparison studies of MDR- and XDR-TB (involving more than 5,000 patients) found a significant association between surgical intervention and successful outcome when compared to non-surgical treatment alone (OR 2.24, 95% CI: 1.68-2.97) (77). Sub-group analyses of studies involving XDR-TB patients revealed an even more pronounced treatment effect (OR 4.55, 95% CI: 1.32-15.7), which would support the widely held view that surgery as a therapeutic option becomes even more attractive as effective chemotherapeutic options dwindle.

Conclusions

MDR-TB, XDR-TB, and now resistance beyond XDR-TB (TDR or XXDR-TB) are growing epidemics fuelled by failing national TB programs, HIV co-infection, and poverty, and not only have a high mortality but threaten to destabilise many national TB programs. For example,

in South Africa, despite drug-resistant TB forming less than 3% of the total case load, it consumes over 40% of the ~$160 million national TB program budget. There is also the growing problem of therapeutic failures who, because of lack of appropriate alternative facilities, are now being discharged back into the community (78). Although surgery remains a critical part of management, only a small number of patients are amenable to surgery. In TB endemic countries, where it is needed most, qualified thoracic surgeons and surgical facilities are lacking. Even where thoracic facilities are available, there may be hesitation and reluctance amongst some thoracic surgeons to operate on patients with a deadly disease. Even with surgery, outcomes in TB endemic countries are poor. New TB drugs are urgently needed, however, policy makers face an ethical dilemma of making these available as part of drug-sensitive regimens, thus preserving their medium to long term efficacy, or risking shortening the effective lifespan of these drugs by using them first in those with DR-TB. Newer and less costly point-of-care diagnostics for drug-resistant TB are also needed, as is an effective TB vaccine. Above all, however, existing national TB programs need to be strengthened so that drug-resistant TB is prevented. In parallel, the existing case burden needs to be tackled and ongoing transmission minimised.

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The multidrug-resistant tuberculosis threat: old problems and new solutions

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With more than 1 million deaths and 9 million estimated incident cases in 2013 tuberculosis represents one of the top clinical and public health priorities worldwide (1).

The World Health Organization (WHO) public health strategy known as DOTS, launched in the 90s' and then scaled-up globally, has contributed to revert the TB incidence trend and the disease-related mortality, if the current estimates are compared with those of the pre-DOTS era. However, TB control and eventually elimination (i.e., defined as less than one case per million population) are challenged by the cursed duet (the TB/HIV co-infection) and by multidrug-resistant tuberculosis (MDR-TB, tuberculosis caused by *Mycobacterium tuberculosis* strains resistant to at least two of the most powerful first-line anti-TB drugs, isoniazid and rifampicin).

The WHO estimated that 13% of the 9 million TB cases (1.1 million people) were HIV-infected, particularly in the WHO African Region, where 4 out of 5 of these cases are estimated to occur in 2013. The qualitative and quantitative immune-deficiency related to the HIV replication, not controlled by appropriate anti-retroviral therapy, and significantly increases the probability of acquiring TB disease given infection. In these individuals the clinical course of pulmonary and extra-pulmonary TB is more severe and associated with higher probability of death.

Furthermore, MDR-TB (which largely is a man-made phenomenon) has become a serious problem in those geographical areas where national health-care systems face economic, logistic, and organizational weaknesses (e.g., in Africa, Asia, and former Soviet Union countries) (2,3). The 2014 WHO Global Report estimated that 3.5% and 20.5% of new and previously treated TB cases had MDR-TB, with a total estimated incidence of 480,000 cases in 2013. Unfortunately, only 136,000 of them were detected, so that transmission of drug resistant strains is rampant among close contacts, treatment with inappropriate anti-TB regimens further increasing the probability of developing additional antibiotic resistances (1).

Aim of the present contribution is to describe the global MDR-TB context, focusing on the problems related to its prevention, diagnosis and treatment. In particular the new and re-proposed drugs are described into details. The review of the literature, although non-systematic, has been done to capture the recent key articles available.

Managing MDR: lights and shadows

The best way to prevent MDR-TB is to diagnose and treat correctly the cases whose strains of *Mycobacterium tuberculosis* are susceptible to first-line anti-TB drugs. A recent European study demonstrated that clinical and public health mismanagement of MDR-TB cases occurred also in highly specialized reference centers of the European Union. Management pitfalls were detected mainly in infection control activities (implementation of administrative and environmental measures), as well as inappropriate

use of personal protective equipment), prescription of anti-TB drugs (particularly dosage and duration of second- and third-line antibiotics) and anti-retrovirals, as well as contact tracing of close contacts (4,5).

In low- and middle-income countries the situation is even worse due to the sub-optimal laboratory network (not making systematic culture, drug susceptibility testing and rapid molecular testing with Xpert MTB/RIF possible) and second line anti-TB drugs (6). Treatment of MDR-TB cases is long, expensive and complicated by the frequent occurrence of adverse events (AE), calling for changes in the prescribed regimen and need for ancillary drugs. All this may compromise the patients' adherence to treatment.

In 2013, 97,000 patients were administered an appropriate treatment for MDR-TB. Although this represents a low proportion if compared with the estimated prevalent cases worldwide, the positive news is that the number of treated cases is today higher than those treated in 2009 (1).

Unfortunately more and more severe cases of MDR-TB, known as extensively-drug resistant tuberculosis (XDR-TB) (i.e., TB caused by MDR *Mycobacterium tuberculosis* strains resistant to any fluoroquinolones and to one of the second-line injectable drug amikacin, kanamycin, and capreomycin) have been described. The XDR-TB definition was considered to have a clinical and operational value (7) because of its worse clinical appearance coupled with worse prognosis. More recently, the new acronym totally drug-resistant tuberculosis (TDR-TB) was proposed for the top severe cases, but this definition was not endorsed by WHO because the drug-susceptibility testing for third-line drugs is not reliable and a complete characterization of the anti-TB drugs which should be *ex vivo* tested to ascertain their susceptibility is missing (8). The WHO estimated that the proportion of MDR-TB with an XDR-TB form should be 9% (1).

A systematic review and an individual patient data meta-analysis carried out a few years ago collected the most relevant MDR-TB cohorts described in the scientific literature (32 datasets including 9,153 patients) from 23 countries belonging to all WHO Regions, in order to assess the clinical outcomes and the covariates associated with the best success rates (9). Fifty-four percent, equivalent to 4,934 patients, achieved treatment success (cure or treatment completion), whereas 23% defaulted, 15% died, and 8% failed or relapsed. Individuals who defaulted or died were older, HIV-infected, previously exposed to anti-TB drugs, and with a more severe disease.

The administration of ethionamide/prothionamide,

as well as ofloxacin or late-generation quinolones, pyrazinamide, and cycloserine was associated with a higher probability of treatment success, compared to failure, relapse and death. Unfortunately, the prescription of group 5 drugs (amoxicillin-clavulanate, clofazimine, clarithromycin, and thiacetazone), whose efficacy is unknown for *Mycobacterium tuberculosis*, did not demonstrate higher effectiveness in a subset of patients belonging to the pooled cohort. The treatment success rate was higher in those treated with at least four and three drugs during the intensive and the continuation phase, respectively. The effective duration of the intensive phase should be from 7 to 8.4 months, whereas the effective duration of the therapy should be 24.6-27.5 months.

The same authors proved how additional resistances to a basic MDR-TB pattern could significantly influence the treatment success rate: it was 64% for patients infected by mycobacterial strains with resistances only to isoniazid and rifampicin, whereas decreased in cases with further resistance to second-line injectable drugs (56%), fluoroquinolones (48%), or to second-line injectable drugs and fluoroquinolones (40%). The latter group (XDR-TB) could be successfully treated if exposed to at least six and four anti-TB drugs during the intensive and the continuation phase, respectively. Moreover, the duration is also important, as an intensive phase of 6.6-9 months and a total duration of 20.1-25 months proved to be successful. Additional resistances to the XDR pattern (for instance, group four drugs) were associated with higher rates of death and treatment failure (10,11).

Two new drugs after 40 years of neglect

The current WHO guidelines for MDR-TB (12), based on the above-mentioned systematic review and an individual patient data meta-analysis, support the best possible use of old, toxic, and poorly effective drugs. When facing XDR- or TDR-TB cases clinicians have difficulties in identifying a sufficient number of anti-TB drugs to design an effective regimen. This was a situation pushing them back to a pre-antibiotic era (13).

In this context, the need of new anti-TB drugs is obvious. After more than 40 years without new TB drugs, two new drugs (bedaquiline and delamanid) have been recently approved by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) for the American and European market, respectively (*Table 1*).

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Table 1 Summary of the main studies on bedaquiline and delamanid

Drug/registration number	Class	Study ID number/ clinical trial phase	Main findings	References
Bedaquiline (TMC207)/ NCT00449644	Diarylquinoline	TMC207-TIDP13-C208/phase II	The addition of bedaquiline (TMC207) to OBR reduced the time to C conversion *vs.* OBR from 125 to 83 days (HR 2.44; 95% CI, 1.57-3.80; P<0.001) and increased the proportion of C converters at 24 weeks (79% *vs.* 58%; P=0.008) and at 120 weeks (62% *vs.* 44%; P=0.04). Cure rates at 120 weeks were 58% and 32% respectively (P=0.003). The incidence of AE was similar in group and control patients but ten and two unexplained deaths were registered, respectively	(14)
Bedaquiline	Diarylquinoline	Compassionate use programme	The article reports the first two cases treated with bedaquiline under compassionate use. No AE were noted	(15)
Delamanid Trial 204 (OPC-67683)/ NCT00685360	Nitroimidazole	242-07-204/phase II	Among patients who received OBR plus 100 mg of delamanid BID, 45.4% had C conversion at 2 months *vs.* 29.6% OBR patients (P=0.008). As compared with OBR, the group receiving OBR plus delamanid 200 mg BID had a higher proportion of SS and C conversion (41.9%, P=0.04). Most AE were mild to moderate and evenly distributed across groups. Although no clinical events due to QT prolongation on ECG were observed, QT prolongation was reported significantly more frequently in the delamanid groups	(16)
Delamanid Trial 208 (OPC-67683)/ NCT01424670	Nitroimidazole	242-09-213/phase III	Patients who participated in the previously reported controlled trial of delamanid and the subsequent open-label extension trial were eligible to participate in a 24-month observational study designed to capture treatment outcomes. Favorable outcomes were observed in 143 (74.5%)/192 patients receiving delamanid for ≥6 months *vs.* 126 (55%)/229 patients receiving delamanid for ≤2 months. Mortality was reduced to 1.0% among those receiving long-term delamanid *vs.* short-term/no delamanid (8.3%; P=0.001). Treatment benefit also seen among XDR-TB patients	(17)
Delamanid	Nitroimidazole	Compassionate use programme	The article reports the first case treated with delamanid under compassionate use, being a pediatric case. The case was diagnosed in the context of a complex epidemic in Milano, Italy. No AE were noted	(18)

QT, QT interval is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. OBR, optimized background regimen; HR, hazard ratio; CI, confidence interval; SS, sputum smear; AE, adverse events; BID, bis in die (twice daily); ECG, electrocardiogram; XDR-TB, extensively drug-resistant tuberculosis.

Bedaquiline (TMC207), a diarylquinoline, was the first drug released in the market after a clinical development process proving its efficacy, safety, and tolerability (19). In particular, the clinical trials confirmed its ability to inhibit the *Mycobacterium tuberculosis* ATP synthase and, consequently, its replication. The initial bacteriostatic action is followed by a bactericidal effect after 5-7 days. Phase II studies demonstrated positive pharmacological interactions with the other first- and second-line anti-TB drugs. The proportion of culture converters was high and the probability of developing resistance to bedaquiline was low. However, experts highlighted that its future efficacy is strictly related to the prescription of an appropriate backbone regimen as per WHO guidelines (proper drug selection, duration and dosages, based on full drug-susceptibility testing) (14,20-22). The drug has been initially available under compassionate use, and proved to be safe (15).

Delamanid (OPC-67683), a nitro-dihydro-imidazooxazole, is able to inhibit the synthesis of the mycolic acid, removing a crucial component of the bacterial wall. Its bactericidal activity has been compared to that of isoniazid, acting in both the intra- and extra-cellular environment (23). One study showed that delamanid-containing regimens could significantly decrease the mortality after 6 months of treatment. The delamanid-related treatment success was 74.2% in a large cohort of MDR-TB patients after 6 months of treatment; furthermore, its efficacy was also confirmed in the XDR-TB group (treatment success: 61.4%) (16,17,24). The first case treated under compassionate use was a pediatric case, who tolerated the treatment well (18).

Knowing more on re-proposed drugs

In parallel with the studies on bedaquiline and delamanid, other observational and experimental trials were conducted to assess efficacy, safety, and tolerability of other antibiotics licensed to treat different bacterial infections. In particular, linezolid (used to treat methicillin-resistant *Staphylococcus aureus* infections) and glycopeptides (vancomycin and teicoplanin) are used off-label to treat MDR- and XDR-TB patients when the therapeutic alternatives do not allow designing an effective regimen (25-29). A systematic review and individual patient data meta-analysis collected all the global observational experience on linezolid to treat drug-resistant *Mycobacterium tuberculosis* strains and pooled individual data to provide a clear picture on its efficacy, safety, and tolerability (25). The linezolid-containing regimens prescribed to

121 MDR-TB patients contributed to achieve high sputum smear (SS) and culture conversion rates (92.5% and 93.5%, respectively). The pooled success rate was 81.8%. If the efficacy was similar comparing outcome indicators in individuals treated with a daily dosage >600 or ≤600 mg, the safety and tolerability profiles were significantly better in those treated with a lower daily linezolid dosage. The most relevant AE involved the blood count (i.e., anemia and thrombocytopenia), the peripheral nervous system, the optic nerve, and the gastro-intestinal apparatus. Those findings confirmed the results of the largest observational, multi-country cohort which underscored the potentiality of this drug (26). The validity of the retrospective observational design was confirmed by the first experimental study which enrolled 41 XDR-TB patients (27,28). The majority of them (87%) achieved sputum culture conversion after 6 months of linezolid exposure. However, potentially linezolid-related AE occurred in 82% of cases, leading to its permanent discontinuation in three individuals. All the XDR-TB cases were treated with 600 mg once daily. A sub-group of individuals treated with 300 mg per day proportionally experienced less AE. The assessment of the blood concentration of linezolid [the so called Therapeutic Drug Monitoring (TDM)] is able to reduce the side effects related to highest concentration of the drug, while maintaining adequate concentration and achieving economic savings, given linezolid is very expensive (30).

Another interesting class of antibiotics, used off-label in difficult-to-treat cases is represented by the carbapenems supported by a β-lactamase inhibitor. Meropenem showed interesting preliminary results in the only study published as of today. This observational, retrospective study addressed the issues related to meropenem efficacy and safety; it was administered with linezolid to 37 cases in Italian and Dutch centers at a daily dosage of 3 g. Although patients treated with meropenem had a more severe disease, the proportion of SS and culture conversion was lower in individuals exposed to a linezolid-containing regimen only in comparison with those treated with meropenem. One patient only discontinued meropenem because of liver toxicity (31). Very recently our group published the first report on the use of ertapenem at the dose of 1 g daily during the ambulatory phase of treatment. The drug was well tolerated (32).

The tolerability and pharmacokinetic profile of co-trimoxazole, frequently prescribed for different bacterial, fungal, and parasitic infections, was proven in a study enrolling ten patients. The Authors concluded that the drug

was effective and safe, a single patient reporting gastrointestinal symptoms (33).

The role of surgery

Surgical intervention was perceived as an old-fashioned therapeutic option although it has been frequently used in some geographical areas (e.g., in former Soviet Union countries) as an alternative or an adjunctive therapy for TB patients. However, more, recently surgery has been re-discovered to manage difficult-to-treat MDR-/XDR-TB patients (34-36). A systematic review and meta-analysis showed that the success rate in those surgically treated is higher [with odds ratio (OR) >2], particularly in the XDR-TB patients (OR of treatment success: 4.55) (34). The WHO Regional Office for Europe issued a document, supported by experts, where observational scientific evidence proved the efficacy of surgery in localized forms of MDR- and XDR-TB, particularly after the patient's exposure to 4-6 active anti-TB drugs. However, the expert panel recommended several conditions allowing surgical interventions, which include good pulmonary reserve, localized forms, low surgical risk, and proven previous failure with polichemotherapy. The interventions should be performed only in specialized centers with an appropriate infection control system in place (35,36).

Conclusions

The MDR-TB phenomenon has been defined a 'back to the future'; it need to be adequately addressed. The WHO's Stop TB Strategy, launched in 2006, underlined for the first time the risk posed by the MDR-TB and TB/HIV co-infection pandemics to the global TB control and provided strategic answers to tackle them. The new End TB Strategy, supporting the vision of TB elimination, further elaborates on how to prevent its occurrence while managing the cases already existing.

The availability of two new drugs and of several re-proposed ones, gives us a real opportunity. Day after day we understand better how to use these new therapeutic options both when added to an optimized background regimen (OBR) or when included in new regimens.

It is important to underline that an essential condition to protect the new drug is to ensure their rational use within stronger public health systems supporting patient's adherence and preventing default and failure (37,38). Without governments' commitment and adequate funding we risk losing in a few years the results of 40 years of efforts.

Acknowledgements

Footnote

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Tuberculous pleural effusions: advances and controversies

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Abstract: On a global scale, tuberculosis (TB) remains one of the most frequent causes of pleural effusions. Our understanding of the pathogenesis of the disease has evolved and what was once thought to be an effusion as a result of a pure delayed hypersensitivity reaction is now believed to be the consequence of direct infection of the pleural space with a cascade of events including an immunological response. Pulmonary involvement is more common than previously believed and induced sputum, which is grossly underutilised, can be diagnostic in approximately 50%. The gold standard for the diagnosis of tuberculous pleuritis remains the detection of *Mycobacterium tuberculosis* in pleural fluid, or pleural biopsy specimens, either by microscopy and/or culture, or the histological demonstration of caseating granulomas in the pleura along with acid fast bacilli (AFB). In high burden settings, however, the diagnosis is frequently inferred in patients who present with a lymphocytic predominant exudate and a high adenosine deaminase (ADA) level, which is a valuable adjunct in the diagnostic evaluation. ADA is generally readily accessible, and together with lymphocyte predominance justifies treatment initiation in patients with a high pre-test probability. Still, false-negative and false-positive results remain an issue. When adding closed pleural biopsy to ADA and lymphocyte count, diagnostic accuracy approaches that of thoracoscopy. The role of other biomarkers is less well described. Early pleural drainage may have a role in selected cases, but more research is required to validate its use and to define the subpopulation that may benefit from such interventions.

Keywords: Pleural effusion; tuberculosis (TB); thoracentesis; pleural biopsy

Introduction

Mycobacterium tuberculosis (*M. tuberculosis*) is a pathogenic bacterial species in the family Mycobacteriaceae and the causative agent of most cases of tuberculosis (TB) (1). Despite being isolated by Robert Koch in 1882, as well as the availability of effective treatment and the use of a live attenuated vaccine in many parts of the world, TB remains one of the deadliest communicable diseases. In 2013, an estimated 9 million people developed active TB, with 1.5 million deaths attributed to the disease (2). According to the World Health Organisation the incidence of pulmonary TB in some regions is as high as 1,000 cases per 100,000 persons (2). Although TB affects the lungs in the majority of patients, extrapulmonary TB serves as the initial presentation in about 25% of adults, and primarily involves

the lymph nodes and pleura (3). This review gives an overview of the pathogenesis, clinical presentation, diagnosis and treatment of TB pleural effusions, highlighting recent advances and controversies.

Pathogenesis

Until recently TB pleural effusions were thought to occur largely as a result of a delayed hypersensitivity reaction. Injecting tuberculin into the pleural cavity of guinea-pigs sensitized with heat killed *M. tuberculosis*, produces a large protein-rich pleural effusion over a 24-hour period, which is completely suppressed by antilymphocyte serum (4). Based on this model and the fact that researchers were unable to culture *M. tuberculosis* from pleural fluids, the pathogenesis was presumed to be due to delayed hypersensitivity rather

than a direct infection of the pleural space. With the advent of improved culture media it is now possible to culture *M. tuberculosis* from both pleural fluid and pleural tissue in as many as 70% of cases (5), and following Koch's postulate for infection, this would suggest a causal relationship (6).

The pleural effusion is likely a manifestation of paucibacillary mycobacterial infection within the pleural space, which is acquired from initial parenchymal lesions and results in an immunological response that both increases pleural fluid formation and decreases pleural fluid removal (7). Initially, there is a rapid neutrophilic inflammatory response within the pleura which is symptomatic. This is followed by a protracted lymphocyte driven immune reaction which is accompanied by pleural granuloma formation and release of adenosine deaminase (ADA). It is therefore plausible that the likelihood of a positive pleural fluid culture decreases with time, as the effusion becomes lymphocyte predominant, and viable mycobacteria are contained.

Similar to lung parenchymal TB, the pathogenetic hypothesis of pleural TB suggests that a strong T-helper type 1 (Th1)-like immunity (interferon dominant) is essential for the containment of *M. tuberculosis*, while these protective effects are antagonized by T-helper type 2 cytokines, primarily interleukin (IL)-4 (8). Activated CD3+ and CD4+ Th1 cells, through the release of interferon gamma (IFN-γ) and other Th1 cytokines, activate macrophages to kill *M. tuberculosis*, whereas Th2 cytokines may antagonize this effect (9). The predominance of Th1 immunity in TB pleural effusions is confirmed by the high levels of IFN-γ, and other inflammatory cytokines (e.g., IL 12), while the proportion of helper T-cells in pleural fluid are also elevated compared with serum or peripheral blood (8,10,11), thus creating a compartmentalised pleural space. The frequency of IL-4 producing T-cells, representing Th2 immunity, is significantly lower in pleural fluid compared to peripheral blood (8). This compartmentalisation may not occur immediately after infection, as shown by animal and *in vitro* studies (12,13). Polymorphonuclear leukocytes are the first cells to respond, remaining the predominant cells for the first 24 hours, and are then followed by macrophages, which peak at 96 hours, and then by lymphocytes. It seems the polymorphonuclear leukocyte influx is a specific response to pleural injury and, either through itself or its interaction with the macrophage, plays a role in host defence mechanisms against the tubercle bacilli (12,13).

Clinical manifestations

Pleural is second only to lymphatic involvement as a site of

extrapulmonary TB (7,14), and may occur in either primary or reactivation disease (15,16). In the USA 3-5% of TB patients are reported to have pleural disease (14), while the incidence of pleural involvement may be as high as 30% in high-burden TB settings (17,18). Where HIV is endemic, TB pleuritis is the most common cause of a lymphocytic effusion and thought to be caused by primary infection in 30% of patients (18). Most (HIV negative) patients with TB pleural effusions will present with an acute febrile illness characterised by a non-productive cough and pleuritic chest pain, but without an elevation in the peripheral white blood cell count (7,19). Night sweats, chills, weakness, dyspnoea, and weight loss are also frequently reported (20). TB pleural effusion may resolve spontaneously without treatment, but patients frequently develop active TB at a later date (21). In non-HIV endemic areas where reactivation is the predominant mechanism of TB disease, pleural involvement is reported to occur in 4% of cases. Such patients have a more insidious onset, are of older age and are more likely to be immunocompromised (18). Immunocompromised patients have been shown to have higher positive culture rates, thought to be due to impaired clearing of the organism (22).

Chronic TB empyema is less common, and represents a distinct entity of chronic, active infection within the pleural space. It is characterised by purulent fluid where virtually all the nucleated white blood cells are neutrophils, and can occur in several settings: (I) progression of a primary TB pleuritis; (II) direct extension of infection into the pleural space from thoracic lymph nodes or a subdiaphragmatic focus; (III) haematogenous spread; or (IV) following pneumonectomy (9). The majority of empyemas will resolve leaving a thickened, scarred, and calcified pleura (23). However, this process may be complicated by decompression through the chest wall (empyema necessitans) (24). Pneumothorax secondary to TB often heralds severe pulmonary involvement by the infectious process and the onset of bronchopleural fistula and empyema (23).

Pleural fibrosis or fibrothorax (*Figure 1*) is a well described complication of TB pleuritis (25). However, uncertainty remains to the exact prevalence, with reports varying between 5% and 55% (26,27). Further, pleural fibrosis may have long term clinical implications, with some studies reporting the association of residual pleural thickening (≥10 mm) with significant morbidity, including chronic chest pain, dyspnoea as well as impairment in lung function (25,28).
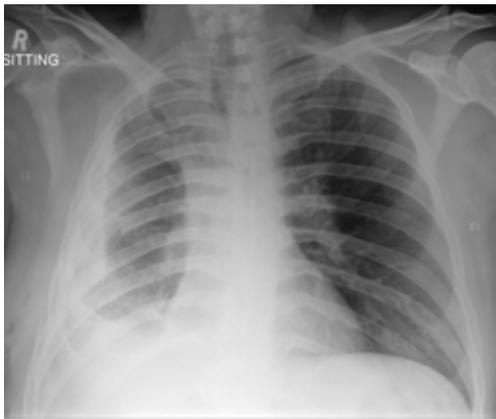
Figure 1 This chest radiograph demonstrates a severe case of fibrothorax as a long-term complication of TB pleuritis. TB, tuberculosis.

Figure 2 This chest radiograph was obtained from a patient with confirmed pleural TB. No significant parenchymal changes could be observed. TB, tuberculosis.

Chest X-ray

Pleural effusions secondary to TB are largely unilateral with a slight right-sided predominance, reported to occur in 55% of cases (*Figure 2*) (29). The effusions are typically small to moderate in size, occupying less than one-third of the hemithorax in approximately 80% of cases (29). However, neither the size nor side of the effusion has been reported to have bearing on prognosis (30).

The coexistence of parenchymal disease in association with pleural effusion has been observed on chest radiograph

Figure 3 A CT from a patient with confirmed TB pleural effusion with parenchymal involvement (sub-segmental consolidation in this particular case). CT, computed tomography; TB, tuberculosis.

in up to 50% of patients (7), and occurs on the same side in almost all cases (20). Observed parenchymal changes are located in the upper lobes in three quarters of cases, suggesting reactivation as the cause of TB. In the remaining patients, parenchymal disease is located in the lower lobe suggesting primary TB infection (20).

Ultrasonography

Thoracic ultrasound is now accepted as standard of care in performing thoracentesis and closed pleural biopsies (31). Detection of localised pleural thickening and other pleural abnormalities can direct the operator to a preferred biopsy site (32). However, apart from the performance of invasive diagnostic procedures, ultrasound may additionally assist in characterising the nature of the effusion (31). The ultrasonographic appearance of pleural effusions secondary to TB range from anechoic to complex septated or non-septated to even homogeneously echogenic effsuions (33,34).

Computed tomography (CT)

CT of the chest is currently the best imaging modality to visualise both pleura and lung parenchyma in TB pleural effusions (*Figure 3*). Apart from visualising the extent of the disease, CT can be used to assess TB empyema, which can be divided into three distinct phases. The exudative phase represents the initial uncomplicated effusion, followed by the fibrinopurulent phase, where CT typically shows thickened visceral and parietal pleurae separated by fluid, known as the "split pleura" sign. In the organizing phase, CT reveals a loculated pleural fluid collection with a

thickened pleural peel and variable degree of calcification with or without proliferation of extrapleural fat (24).

CT is more sensitive in diagnosing coexistence of parenchymal abnormalities when compared to conventional chest radiographs. In a recent study, more than 80% of patients had pulmonary parenchymal abnormalities when CT scanning was performed (35). The most common CT findings being micronodules in both the subpleural and peribronchovascular interstitium, with interlobular septal thickening, suggesting lymphatic spread of TB (35). Interestingly, in addition to these findings, a paradoxical response to treatment is not rare in patients with pleural TB, and is noted especially in young, previously healthy, male patients with subpleural nodules on initial CT scans (35).

Diagnosis

Principles

The gold standard for the diagnosis of TB pleuritis is detection of *M. tuberculosis* in the sputum, pleural fluid or pleural biopsy specimens, either by microscopy and/ or culture, or the histological demonstration of caseating granulomas in the pleura along with acid fast bacilli (AFB) (18,28,34). In high burden settings, however, the diagnosis is frequently inferred in patients who present with a lymphocytic predominant exudate and high ADA level.

Sputum

It was previously believed that patients without overt coexisting parenchymal lesion are sputum negative and, therefore, noncontagious. Moreover, the mycobacterial culture rate in non-induced sputum is low. Reported sensitivities range from 0% to 30% (36). The method of sputum collection, however, is of crucial importance. Conde *et al.* (37) reported a yield of 52% on mycobacterial culture with induced single sputum specimens. Even in patients with normal underlying lung parenchyma on chest X-ray, the yield of sputum culture in induced samples approached 55%. Therefore, in patients with suspected TB pleural effusion it is important to obtain cultures on induced sputum samples, even in the absence of obvious parenchymal involvement. To our knowledge there are no studies evaluating the role of sputum nucleic acid amplification (NAA) molecular studies such as Xpert MTB/ RIF in the context of TB pleural effusions. NAA tests may increase the diagnostic yield of sputum compared with conventional microscopy, but is unlikely to be more sensitive than culture.

Thoracentesis

Microscopy and culture

The macroscopic appearance is that of straw coloured fluid in more than 80% of cases (5). Microscopy for AFB in the pleural fluid can identify *M. tuberculosis* in fewer than 10% of cases. The exception to this is patients with HIV and tuberculous empyema, where yields may be higher (>20%) (9). Culture of the pleural fluid can be performed on either solid or liquid media as in the commercially available and widely used BACTEC MGIT semi-automated system (Becton-Dickinson, Franklin Lakes, NJ, USA), or with manual culture methods that might allow resistance testing at the same time such as the microscopic-observation drug susceptibility (MODS) assay (38). When using solid culture media, sensitivities reported have been low, in the range of 12% to 30%. However, it appears that liquid culture media display better sensitivities of up to 70%. A further benefit of using liquid media is the significantly shorter time required for culture results, being 2 weeks compared to the traditional 6 weeks for solid media (39).

Perhaps counter-intuitively, it does not appear that sending larger volumes of pleural fluid for culture improves diagnostic sensitivity. von Groote-Bidlingmaier *et al.* compared the yield of high and low pleural fluid volumes (100 *vs.* 5 mL) inoculated into liquid culture medium in patients with a high pre-test probability of TB (22). It was shown that the absolute yield was not significantly higher for the larger volume (53.5% *vs.* 50% respectively; P=0.75). In the same study, HIV-positive individuals had culture-positive pleural fluid more frequently than HIV-negative patients. This is presumably due to impaired bacterial clearance from the pleural space. Underlying immunosuppression as a contributor is corroborated by the known negative association of lymphocyte percentage in pleural fluid with the probability of positive culture of pleural fluid (5). The combination of pleural fluid and sputum cultures in the diagnostic workup of TB pleuritis seems a reasonable initial approach, with a combined diagnostic yield of almost 80% (5,22). In a recent study the reported diagnostic yield was 63% for effusion culture, 48% for sputum culture and 79% for the combination of effusion and sputum cultures, using liquid culture media (5).

Routine chemistry

The effusion is uniformly exudative having protein concentrations invariably >50, and >30 g/L in 50% to 77% of cases (40). The pleural fluid lactate dehydrogenase (LDH) level is elevated in approximately 75% of cases, with levels commonly exceeding 500 IU/L (7,20,40). The pleural fluid pH is usually less than 7.40 with values below 7.30 in about 20% of cases (20,40). A low pH and a low glucose concentration may be observed but are more characteristic of chronic tuberculous empyema than TB (41,42). In fact, a pleural fluid pH of <7.20 indicates a possible empyema, prompting the physician to consider drainage of the fluid. The pleural fluid glucose concentration in TB is normally between 3.3 and 5.6 mmol/L, with glucose levels <2.8 mmol/L seen in 7-20% of effusions, while extremely low glucose concentrations (<1.7 mmol/L) may occasionally be observed (20,40).

Adenosine deaminase (ADA)

ADA levels are most useful when there is a moderate to high suspicion of TB in patients with negative pleural fluid or biopsy cultures, and non-diagnostic histology (43). There is a wide range of cut-off values used by authors but in the majority of studies the most accurate threshold was found to range between 40 and 60 U/L (43). In a study of 254 patients with pleural TB, 99.6% had ADA more than 47 U/L (29) and in another group of 303 patients in a high TB prevalence population with exudative effusions, 58% had TB with a lymphocytic predominant effusion and ADA more than 50 U/L (44). The diagnostic usefulness of ADA depends not only on its sensitivity and specificity, but also on the local prevalence of TB. In populations with a high prevalence of TB and clinical suspicion of TB effusion, elevated ADA level might be considered as a confirmatory test justifying treatment initiation. In contrast, in countries with a low prevalence of TB, the negative predictive value remains high even though the positive predictive value of pleural ADA declines. This was illustrated by two studies done in populations with low TB prevalence and non-TB lymphocytic effusions, in which 97% and 98% had ADA levels less than 40 U/L (45,46). Therefore, a negative ADA test may justify abandoning further diagnostic procedures for TB, and pursuing alternative diagnoses.

When interpreting ADA levels, the clinician must additionally be aware of situations which may increase the likelihood of both the false-negative and false-positive ADA results. In the early phase of the disease low levels of ADA in the pleural fluid may be found, giving rise to a false negative result. However, ADA levels will invariably be elevated if thoracentesis is repeated a few days later (47). Additional care should also be taken when interpreting pleural ADA levels in elderly patients and/or current smokers, as ADA levels may be low in such TB patients (48). Conversely, raised ADA levels may be observed in a number of conditions potentially leading to a false positive diagnosis of TB. These include rheumatoid effusion, empyema due to other bacteria, mesothelioma, lung cancer, parapneumonic effusion, and haematological malignancies (47,48).

The diagnostic accuracy of ADA can be improved by measuring different ADA isoenzymes. ADA-2 is increased in TB effusions, while ADA-1 is increased in other bacterial empyemas (49), and distinguishing between these two principal isoenzymes can increase the specificity of ADA for diagnosing TB. Use of the ADA-2 isoenzyme measurement increased the specificity for TB from 91% to 96% (50) and 92.1% to 98.6% (51), in two different studies.

Although it has been suggested that ADA might be a less sensitive marker of TB in immunocompromised patients, there is currently little evidence to support this view. Baba *et al*. (52) demonstrated that ADA is a reliable marker of pleural TB in HIV-positive patients, even for those with low CD_4 counts, while Chung *et al*. (53) confirmed that ADA is an accurate marker in renal transplant recipients.

Nucleated cell count and cytology

The cell count performed usually reveals a nucleated (white) cell count between 1,000 and 6,000 cells/mm^3 (29), and has a T lymphocyte predominance in 60% to 90% of cases (7,20,40). However, the predominant nucleated cell type can vary depending on timing of collection of the pleural fluid. Fluid collected in the first few days may exhibit a neutrophil predominant effusion, while lymphocytes tend to dominate thereafter (12,13). A lymphocytic predominant effusion may be defined as one with more than 75% lymphocytes and/or a lymphocyte to neutrophil ratio more than 0.75 (18). When a lymphocyte neutrophil ratio of 0.75 or greater is used in combination with ADA, the sensitivity, specificity, positive predictive value, negative predictive value, and efficiency for the identification of TB were reported at 88%, 95%, 95%, 88%, and 92%, respectively (44). Other cell types are much less common in TB pleural effusions, with eosinophils being rare unless the patient has had a pneumothorax or haemothorax near the time of pleural analysis (54). The presence of more than 5% mesothelial cells is unusual (55). Cytological examination of the pleural fluid is routinely done, as previously stated, as a malignant

pleural effusion (MPE) may present with a lymphocytic predominant exudate as well as a high ADA.

Additional pleural fluid assays and biomarkers

IFN-γ is an important Th1 cytokine and is important in the host's immune response to mycobacterial infection. Measurement of unstimulated pleural fluid IFN-γ concentration by commercially available enzyme-linked immunosorbent assay kits is considered a useful diagnostic tool to diagnose TB pleuritis (49,56). However, concerns similar to that for ADA around the specificity of the test have been expressed. In one study including 145 patients with TB, an IFN-γ concentration >140 pg/mL had sensitivity and specificity of 94% and 92%, respectively (29), while another study (n=66) of exudative, lymphocytic pleural effusions using a cut-off value of 240 pg/mL, found a sensitivity and specificity of 95% and 96% (57). A meta-analysis which included 22 studies to estimate the diagnostic accuracy of IFN-γ for pleural TB, noted sensitivity and specificity of 89% and 97%, respectively (58), while other series have described lower sensitivities (59). More recently, IFN-γ was compared to ADA in a TB-endemic setting and showed a significantly greater sensitivity than ADA (IFN-γ cut-off: 107.7 pg/mL) (60). Thus, it appears that in a high burden TB setting, IFN-γ may have a similar potential utility to the current use of ADA. However, the test is not yet widely available in clinical practice and important information is not yet available regarding false positives and negatives situations, as well as its utility in low TB burden settings, thus preventing definitive recommendations for its use.

NAA techniques for evaluation of TB pleuritis in HIV-negative patients appear to have high specificity but relatively low sensitivity. In a meta-analysis of HIV-negative patients, NAA tests had relatively low sensitivity (62%) but high specificity (98%) for diagnosing TB (61). The relatively high sensitivity of the Xpert MTB/RIF NAA assay contrasts with the sensitivity and specificity of 25% and 100%, respectively, reported in a series of 20 cases of confirmed TB from a region with high TB prevalence (62). More recently the Xpert assay was tested in a high prevalence HIV/TB setting and the sensitivity and specificity of Xpert MTB/RIF test were 28.7% and 96.6%, respectively, while the respective positive and negative predictive values were 96.1% and 31.1% (63). Xpert MTB/RIF test on pleural fluid does not accurately diagnose pleural TB and therefore cannot be used as an initial evaluation test in patients with suspected pleural TB.

Lysozyme is an enzyme found in the cytoplasmic granules of neutrophils which hydrolyses bacterial cell walls. Pleural fluid lysozyme concentrations are >15 mg/dL in 80% of cases of pleural TB (64,65), with highest concentrations found in empyemas due to both tuberculous and non-tuberculous bacteria (65). However, lysozyme is not specific for infection and concentrations may also be elevated in MPE (64). The pleural fluid: serum lysozyme ratio may be more useful than the absolute value of lysozyme. A ratio >1.2 has been reported to diagnose empyema with a 100% sensitivity for TB pleuritis and 95% specificity (65). However, in a separate study of 276 pleural effusions of heterogeneous aetiology, a threshold ratio of 1.1 was associated with a sensitivity of only 67% with specificity of 90% (64), thus casting doubt on its utility.

Additional biomarkers used in differentiating MPE from TB pleuritis are simultaneous measurements of pleural DcR3 and TNF-sR1 (66), while a further study assessed the specificity and accuracy of combinations of TNF- and ADA2 (67). Other novel biomarkers that may in the future, aid diagnosis of TB pleuritis include: hyaluronic acid, neopterin, leptin and fibronectin concentration in pleural fluid, although there is currently limited data, with variable sensitivities and specificities being reported (68). Urinary lipoarabinomannan ELISA has recently been evaluated for the diagnosis of TB in HIV-infected patients with paucibacillary disease and may also be useful in the diagnosis of pleural TB (69).

Pleural biopsy

The presence of caseating granulomas containing acid-fast bacilli on histological examination of the pleural surface is diagnostic of TB pleuritis (9). The demonstration of acid-fast bacilli is not an absolute requirement; the presence of caseating granulomas in high burden settings is considered adequate (18). Pleural tissue can be harvested either by means of closed pleural biopsies, thoracoscopy or open surgical biopsies (34). Access to thoracoscopy and open surgical biopsies is limited in many parts of the world where TB is endemic and, therefore, if pleural fluid analysis proves inconclusive, closed biopsy is the preferred next investigation. Ultrasound-guided biopsy techniques have gained popularity amongst pulmonary physicians and are becoming the standard of care for obtaining pleural tissue in patients with suspected TB pleuritis. Medical thoracoscopy is reserved for the small number of cases where closed pleural biopsies fail to provide a diagnosis (18,34). Ultrasound-guided pleural biopsy has a diagnostic yield of
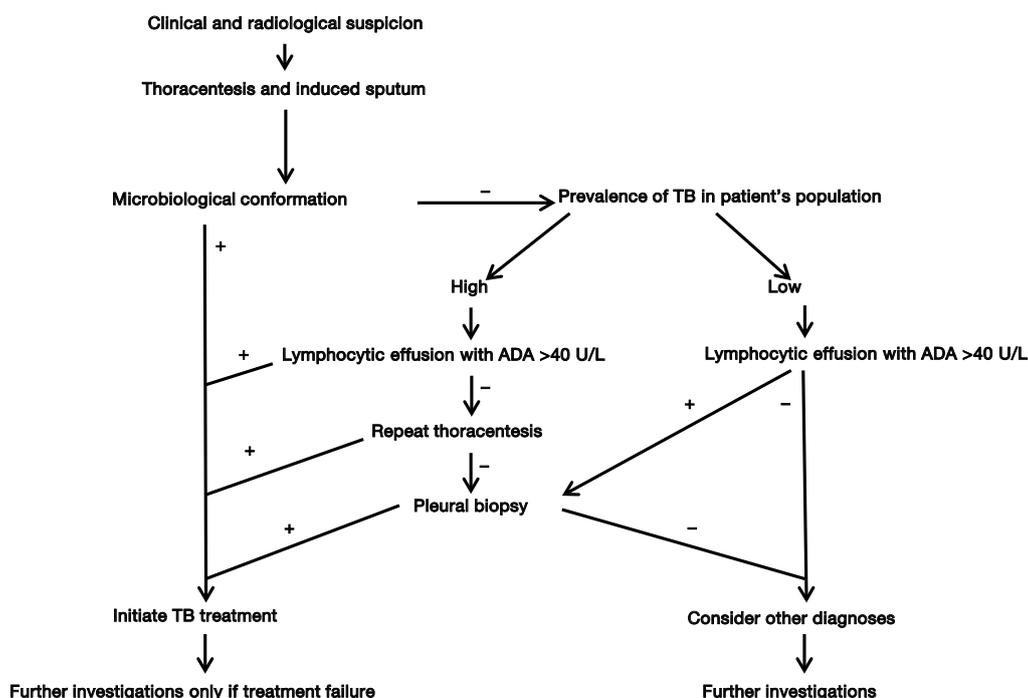
Figure 4 A suggested algorithm for the diagnostic evaluation of a patient who presents with a clinical and radiological suspicion of pleural TB. For the purposes of this approach, a high prevalence of TB is considered to be ≥125 per 100,000 population, whereas a low prevalence is <125 per 100,000. See text for detail on choice of biopsy technique (pleuroscopy *vs.* closed pleural biopsy). TB, tuberculosis.

up to 90% for pleural TB, which is not surprising given the diffuse nature of the disease (22).

Some centres prefer to use the Tru-Cut needle biopsies over the more conventional Abrams needle. Current evidence, however, still favours the Abrams needle as demonstrated by one study which found an overall diagnostic yield of 81.8% for the Abrams needle compared to 65% for the Tru-cut needle (35).

Because of sampling error, sensitivity generally increases with the number of biopsies taken, and biopsies taken during medical thoracoscopy have previously been found to have a diagnostic sensitivity of 100% (34). With medical thoracoscopy direct visualisation of the diseased pleura is possible, using either a rigid or a flexible endoscope, which guides the operator to the most appropriate biopsy site. Due to the reported high yield and less invasive nature of thoracoscopy, open surgical biopsies are seldom required.

Practical diagnostic approach

A practical suggested diagnostic approach, based on pre-

test probability, is presented in *Figure 4*. If the nucleated cell count is lymphocytic predominant with a high ADA (>40 U/L), and the background TB prevalence is high, it is prudent to initiate TB therapy. However, in a low prevalence population further confirmation is required by obtaining pleural biopsies for both histology and culture. Thoracoscopy has a superior diagnostic yield for both pleural malignancy and TB, and is therefore considered by many to be the investigation of choice in exudative pleural effusions where a thoracentesis was non-diagnostic. Pleural fluid findings are known to evolve from early neutrophil to lymphocytic predominance in TB effusions, and the value of repeat thoracentesis has been shown to significantly increase the diagnostic yield. In fact, we have shown that 77.8% of cases of confirmed pleural TB could be diagnosed on a second pleural aspiration in patients who previously had at least one non-diagnostic thoracentesis. In a high burden and resource constrained setting it may therefore be appropriate to repeat ultrasound guided thoracentesis before proceeding to pleural biopsy (70). An image-assisted second thoracentesis combined with an image-assisted

pleural biopsy with either an Abrams needle or cutting needle (depending on the clinical setting and imagery) may be an acceptable alternative to thoracoscopy, particularly when the probability of TB pleural effusion is high.

Treatment

The medical treatment for TB pleural effusion is the same as for pulmonary TB, and is consistent with the theory that the majority of pleural TB cases develop from pulmonary disease. The expected resolution of TB pleural effusion is variable, and assuming appropriate therapy, fever usually resolves within 2 weeks with reabsorption of the pleural fluid within 6 weeks. Naturally this will depend on the burden of disease in the individual, and size of the effusion and resorption may take up to 2-4 months.

The current evidence on the role of surgical intervention is limited and difficult to interpret, and intercostal drainage is traditionally not offered to patients unless severe dyspnoea is present (71,72). A recent study by Bhuniya *et al.* (73) investigated the use of early pleural drainage (using pleural manometry) in addition to standard anti-TB therapy, compared to standard therapy alone; and demonstrated significant differences after 6 months in lung function. The drainage group had a forced expiratory volume in the first second (FEV_1) of 87.6% as compared to the control group of 84.9% (P=0.02), with forced vital capacity (FVC) of 84.5% and 83.3% (P<0.01), respectively. The long term clinical relevance of these small differences in lung function is not known. However, these authors did report a lower incidence of residual pleural thickening in drained patients and also commented that patients with therapeutic thoracentesis experienced immediate relief from dyspnoea after drainage. Earlier studies reported that residual pleural thickening ≥10 mm can cause significant clinical symptoms in patients with TB pleural effusion, with reported incidences varying from 26% to 50.4% (25,26,73,74). In unpublished data, we found that patients with confirmed TB pleural effusions, randomised to therapeutic pleural drainage, showed significantly superior improvements in several lung function parameters after 3 and 6 months follow-up, despite complete drainage being achieved in less than half of all patients.

In selected patients, administration of corticosteroids can shorten the duration of fever and time to fluid resorption, although the risks and benefits of corticosteroids in this setting have not been well defined (75,76). Currently data is insufficient to support routine adjunctive use of corticosteroids for TB pleuritis (77).

Conclusions

On a global scale, TB remains one of the most frequent causes of pleural effusions. Our understanding of the pathogenesis of the disease has evolved and what was once thought to be an effusion as a result of a pure delayed hypersensitivity reaction is now believed to be the consequence of direct infection of the pleural space with a resultant lymphocyte driven immunological response. Pulmonary involvement is more common than previously believed and induced sputum, which is grossly underutilised, can be diagnostic in approximately 50%. The gold standard for the diagnosis of tuberculous pleuritis remains the detection of *M. tuberculosis* in pleural fluid or pleural biopsy specimens, either by microscopy and/or culture, or the histological demonstration of caseating granulomas in the pleura along with AFB. In high burden settings, however, the diagnosis is frequently inferred in patients who presents with a lymphocytic predominant exudate with a high ADA level, which is a valuable adjunct in the diagnostic evaluation. ADA is generally readily accessible, and together with lymphocyte predominance justifies treatment initiation in patients with a high pre-test probability. However, false-negative and false-positive results remain problematic. When adding closed pleural biopsy to ADA and lymphocyte count, diagnostic accuracy approaches that of thoracoscopy. The role of other biomarkers is less well described. Unstimulated pleural fluid IFN-γ or other newer assays, might lead to greater diagnostic accuracy, but further studies are required. Early pleural drainage may have a role in selected cases, but more research is required to validate its use.

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A review of clinical practice guidelines for lung cancer

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Abstract: Clinical practice guidelines are important evidence-based resources to guide complex clinical decision making. However, it is challenging for health professionals to keep abreast available guidelines and to know how and where to access relevant guidelines. This review examines currently available guidelines for lung cancer published in the English language. Important key features are listed for each identified guideline. The methodology, approaches to dissemination and implementation, and associated resources are summarised. General challenges in the area of guideline development are highlighted. The potential to collaborate more widely across lung cancer guideline developers by sharing literature searches and assessments is discussed.

Keywords: Clinical practice guidelines; clinical practice guideline development; evidence-based medicine; lung neoplasms; non-small cell lung carcinoma; small cell lung carcinoma

Introduction

A challenge for health professionals managing patients with lung cancer is to keep abreast with the rapidly growing evidence base in diagnosis, staging and treatment. Clinical practice guidelines for lung cancer provide a useful tool of synthesised evidence to guide complex clinical decision making. They have the potential to enhance the healthcare decisions of clinicians and patients, and to lead to better quality care and improved outcomes for patients when they are of high quality, accessible and successfully implemented (1).

Numerous guidelines have been developed for lung cancer across the world. With a vast number of lung cancer guidelines developed in different countries by different organisations and listed across numerous guideline databases, this review article aims to provide a comprehensive overview of available guidelines for lung cancer available in English language. Key features such as developing organisation(s), publication date, geographic context and access details are listed for each guideline. More detailed information in regards to the methodology, the dissemination and implementation approach, important background information and any associated resources are briefly summarised in the results section.

Methods

Clinical practice guidelines are defined as "statements that include recommendations intended to optimize patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options" (1). This definition has been used to identify clinical practice guidelines for lung cancer to be included in this review article. A comprehensive literature search consisting of searching the Guidelines International Network (GIN) International Guideline Library, National Guideline Clearinghouse, Standards and Guidelines Evidence (SAGE) portal, Australia's Clinical Practice Guideline Portal, PubMed as well as Scottish International Guidelines Network's (SIGN) and National Institute for Health and Care Excellence's (NICE) databases was completed. In addition, snowballing was used to identify any further relevant guidelines that were missed in the database searches. The results were then screened and included if the following criteria were met (*Table 1*).

Guidelines addressing malignant pleural mesothelioma, thymoma, specific symptom management topics and other secondary topics were out of scope for this review article and therefore not considered. Clinical practice guidelines

Table 1 Inclusion criteria
Inclusion criteria
- Clinical practice guideline as per Institute of Medicine's definition (1)
- Published in English language
- Published between 2008 and 2013*
- Addresses prevention, screening, diagnosis, staging, treatment and management of small cell and non-small cell lung cancer
*The date range is based on the maximum guideline validity period of five years according to National Health and Medical Research Council (2).

Table 2 Excluded lung cancer guidelines		
Guideline developer	Guideline title	Reason for exclusion
American Association for Thoracic Surgery (AATS)	The American Association for Thoracic Surgery guidelines for lung cancer screening using low-dose computed tomography scans for lung cancer survivors and other high-risk groups (3).	No explicit information in regards to literature search and review methods included (3).
Central European Cooperative Oncology Group (CECOG)	Third CECOG Consensus on the systemic treatment of non-small-cell lung cancer (4).	Not clear if systematic review was performed (4).
European Society for Medical Oncology (ESMO)	Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (5). Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (6). Small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (7).	Based on a narrative literature search (8).
European Respiratory Society (ERS), European Society of Thoracic Surgeons (ESTS)	ERS/ESTS clinical guidelines on fitness for radical therapy in lung cancer patients (surgery and chemo-radiotherapy) (9).	Based on a non-systematic literature review and expert panel consensus (9).
Italian Association of Thoracic Oncology (AIOT)	Treatment of advanced non-small-cell lung cancer: Italian Association of Thoracic Oncology (AIOT) clinical practice guidelines (10).	Based on a non-systematic literature review and expert panel consensus (10).
National Comprehensive Cancer Network (NCCN)	Lung cancer screening Version 1.2013 (11). Non-small cell lung cancer Version 2.2013 (12). Small cell lung cancer Version 2.2013 (13).	No information included in regards to literature search and review methods (11-13).
Spanish Society for Medical Oncology (SEOM)	SEOM clinical guidelines for the treatment of non-small cell lung cancer: an updated edition (14).	Methodology not included (14).

that met all criteria, but were based on a non-systematic literature review, were excluded from this review as the systematic review requirement according to the clinical practice guideline definition was not met (*Table 2*). Other forms of clinical guidance such as general consensus statements on clinical topics, expert advice, task force reports, health technology appraisal and appropriate use criteria were also excluded.

Results

In total 22 lung cancer guideline documents developed by 12 different organisations were identified as meeting the inclusion criteria (*Table 3*).

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Table 3 Results of available lung cancer guidelines published between 2008-2013

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
Alberta Health Services	Non small cell lung cancer stage I (15)	2011	Canada	online, pdf format	Evidence-based clinical practice guidelines based on a systematic review and/ or systematic recommendation adoption from an existing guideline	Recommendations statements Example: "A lobectomy or greater lung resection is recommended over a sublobar resection for patients with stage I NSCLC who are medically fit for surgery." (15)	http://www. albertahealthservices. ca/1755.asp.
	Non small cell lung cancer stage II (16)	2011	Canada	online, pdf format			http://www. albertahealthservices. ca/1755.asp.
	Non small cell lung cancer stage III (17)	2011	Canada	online, pdf format			http://www. albertahealthservices. ca/1755.asp.
	Non small cell lung cancer stage IV (18)	2011	Canada	online, pdf format			http://www. albertahealthservices. ca/1755.asp.
	Small Cell Lung Cancer - Extensive Stage (19)	2011	Canada	online, pdf format			http://www. albertahealthservices. ca/1755.asp.
	Small Cell Lung Cancer - Limited Stage (20)	2011	Canada	online, pdf format			http://www. albertahealthservices. ca/1755.asp.
American College of Chest Physicians (ACCP)	Diagnosis and Management of Lung Cancer, 3rd ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (21)	2013	USA	Journal article	Evidence-based clinical practice guideline based on a systematic review	Graded recommendation statements according to ACCP's system (21) Example:" In patients with PET activity in a mediastinal lymph node and normal appearing nodes by CT (and no distant metastases), invasive staging of the mediastinum is recommended over staging by imaging alone (Grade 1C)." (21)	http://www.chestnet. org/Guidelines-and- Resources/Guidelines- and-Consensus- Statements/More- Guidelines/Lung-Cancer

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
American Society for Radiation Oncology (ASTRO)	Palliative thoracic radiotherapy in lung cancer: An American Society for Radiation Oncology evidence-based clinical practice guideline (22)	2011	USA	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Guideline statements Example: "At this time, there is no added benefit for the use of chemotherapy concurrently with radiation therapy (RT) in the palliation of thoracic symptoms in lung cancer patients. To date, there is 1 randomized phase III study directly addressing this issue." (22)	https://www.astro.org/Clinical-Practice/Guidelines/Palliative-thoracic.aspx
American Society of Clinical Oncology (ASCO)	2011 Focused Update of 2009 American Society of Clinical Oncology Clinical Practice Guideline Update on Chemotherapy for Stage IV Non–Small-Cell Lung Cancer (23)	2011	USA	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Narrative recommendation statements according to ASCO's system (24) Example: "Evidence supports the use of chemotherapy* in patients with stage IV non–small-cell lung cancer with Eastern Cooperative Oncology Group (ECOG)/Zubrod* PS 0, 1, and possibly 2." (23)	http://www.asco.org/institute-quality/asco-clinical-practice-guideline-update-chemotherapy-stage-iv-non-small-cell-lung
British Thoracic Society (BTS), Society for Cardiothoracic Surgery (SCTS)	Guidelines on the Radical Management of Patients with Lung Cancer (25)	2010	UK	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Graded recommendation statements according to Scottish Intercollegiate Guidelines Network (SIGN) system (25) For example: "View all available historical images at the onset of the diagnostic pathway and review them prior to treatment. [C]" (25)	http://www.brit-thoracic.org.uk/guidelines.aspx

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
Cancer Care Ontario	First-line systemic chemotherapy in the treatment of advanced non-small cell lung cancer (26)	2010	Canada	online, pdf format	Evidence-based clinical practice guidelines based on a systematic review	Recommendations, key evidence and qualifying statements Example: "Combination PET-CT imaging data may be used as part of research protocols in RT planning. Current evidence does not support the routine use of PET-CT imaging data in RT planning at this time outside of a research setting." (27)	https://www.cancercare. on.ca/toolbox/ qualityguidelines/ diseasesite/lung-ebs/
	Positron emission tomography in radiation treatment planning for lung cancer (27)	2010	Canada	online, pdf format			https://www.cancercare. on.ca/toolbox/ qualityguidelines/ diseasesite/lung-ebs/
	Postoperative adjuvant radiation therapy in stage II or IIIA completely resected non-small cell lung cancer (28)	2013	Canada	online, pdf format			https://www.cancercare. on.ca/toolbox/ qualityguidelines/ diseasesite/lung-ebs/
	Use of preoperative chemotherapy with or without postoperative radiotherapy in technically resectable stage IIIA non-small cell lung cancer (29)	2013	Canada	online, pdf format			https://www.cancercare. on.ca/toolbox/ qualityguidelines/ diseasesite/lung-ebs/
	Altered fractionation of radical radiation therapy in the management of unresectable non-small cell lung cancer (30)	2013	Canada	online, pdf format			https://www.cancercare. on.ca/toolbox/ qualityguidelines/ diseasesite/lung-ebs/

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
	Second-line or subsequent systemic therapy for recurrent or progressive non-small cell lung cancer (31)	2012	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	The Role of Combination Chemotherapy in the Initial Management of Limited-Stage Small-Cell Lung Cancer (32)	2012	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Chemotherapy for relapsed small cell lung cancer (33)	2013	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Fluorodeoxyglucose positron emission tomography in the diagnosis and staging of lung cancer (34)	2012	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
Cancer Council Australia (CCA)	Clinical practice guidelines for the treatment of lung cancer (35)	2012	Australia	web-based	Evidence-based clinical practice guidelines based on a systematic review	Graded recommendations and levels of evidence according the NHMRC's system (35) Example:" Patients who have a good performance status (WHO 1, 2) and completely resected stage III non-small cell lung cancer should be offered adjuvant cisplatin-based chemotherapy." (35)	http://wiki.cancer.org.au/australia/Guidelines:Lung_cancer

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), Association for Molecular Pathology (AMP)	Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors (36)	2013	USA	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Graded recommendations including levels of evidence according to the NHMRC's system (36) Example: "ALK molecular testing should be used to select patients for ALK-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics. Evidence Grade: EGFR: A ALK: B…." (36)	http://www.archivesofpathology.org/doi/pdf/10.5858/arpa.2012-0720-OA
National Institute for Health and Care Excellence (NICE)	Lung cancer. The diagnosis and treatment of lung cancer (37)	2011	UK	web-based and pdf version	Evidence-based clinical practice guidelines based on a systematic review	Recommendation statements including qualifying statements (37) Example: "Ensure all patients potentially suitable for treatment with curative intent are offered PET-CT before treatment. [NEW 2011]" (37)	http://guidance.nice.org.uk/CG121

Table 3 shows that there is wide variation in nearly every aspect of guideline development between each of the guidelines. As *Table 3* indicates, the scope varied across the identified guidelines. Some guideline developers, such as NICE (37) or American College of Chest Physicians (ACCP) (21), published their lung cancer guideline as one large document covering all areas of lung cancer from epidemiology, screening, diagnosis, treatment, follow-up to end-of-life care. Others, such as Alberta Health Services (15-20) and College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP) (36), developed more focused guidelines addressing specific area(s) of lung cancer. For example, Alberta Health Services released their lung cancer

guidelines as separate discrete publications and published a guideline for each stage (15-20). Cancer Care Ontario's guidelines are even more specific and address only one or a few closely related clinical questions (26-34).

The guideline development approach also varied between organisations. All included clinical practice guidelines are based on formal systematic reviews to generate evidence-based recommendations. However, the evidence assessment tools, recommendation format and recommendation grading schemes vary (*Table 3*). A few developers even have a procedure in place to evaluate if recommendations from existing guidelines could be formally adopted (38) as opposed to developing de novo guidelines/recommendations.

Whereas all identified guidelines are disseminated and

accessible online, the presentation varied from documents available for download, web-based clinical practice guidelines, guidelines available as published journal articles or a combination of approaches (*Table 3*). Many developers offer printed guideline copies or printed summaries of the recommendations available upon request.

The subsequent section summarises background information in regards to the relevant guideline(s), composition of guideline development group, conflict of interest (COI) management, guideline funding, the methodological as well as dissemination and implementation approach, planned update and any associated resources for the guideline(s) in narrative form under each guideline developer or collaboration of guideline developers. Together with *Table 3*, the summarised information covers the subject areas of the standards identified by the Institute of Medicine for developing trustworthy clinical practice guidelines (establishing transparency, management of COI, guideline development group composition, systematic review, evidence foundations and evidence level ratings, recommendation formulation, external review and updating) (1). Dissemination and implementation approach and any associated guideline resources were added for each guideline as these are key to achieve successful guideline uptake (39).

Alberta Health Services

Introduction
Alberta Health Services is a Canadian health authority that delivers health services in the Canadian province Alberta and develops clinical practice guidelines in oncology. For each stage in non-small cell and small cell lung cancer a separate lung cancer guideline document was produced. They are published as separate PDF publications on Alberta Health Services' website (*Table 3*) (15-20).

Guideline development methodology
Guideline development at Alberta Health Services follows a systematic guideline development approach as detailed in the Guideline Utilization Resource Unit Handbook (40). For each lung guideline, a multidisciplinary working group was recruited. The guideline scope was defined and clinical questions developed. The literature searches were carried out by an in-house knowledge management specialist. All retrieved literature results were screened, assessed and synthesised. Existing guidelines were also searched for in order to evaluate if an existing guideline could be formally adopted. Any retrieved existing guidelines were

formally assessed with the AGREE II instrument to ensure minimum requirements for a good quality guideline were met, before considering the formal adoption or adaption of existing recommendation(s). Guideline recommendations were developed and formulated by the guideline working group members based on the evidence tables and expert clinical interpretation or, if applicable, an existing guideline. Recommendations were formulated in the form of action statements and the reasoning behind the recommendation, including the quality and level of evidence, was added in narrative form. Alberta Health Services did not use a formal grading scheme to assign specific grades to recommendations. The draft guidelines were then open for comment and reviewed by all members of the Provincial Tumour Team. Once the guideline documents were finalised, they were formally endorsed by Alberta Health Services.

COI management
COI statements are included in each lung cancer guideline as well as an overall statement from the developer that each guideline was satisfactorily developed in an unbiased manner (40).

Funding
Each lung cancer guideline document states that there was no direct industry involvement in the production or dissemination of the guideline (40).

Dissemination and implementation approach
The guidelines are published on the Alberta Health Services website. All members are notified when a guideline has been updated or added. Guidelines are presented at the local and provincial tumour team meeting as well as weekly hospital rounds to facilitate uptake (40).

Planned update
Alberta Health Services clinical practice guidelines are reviewed and updated every one to two years (40).

Associated resources
Treatment algorithms for each lung cancer guideline are available from http://www.albertahealthservices.ca/1755.asp.

American College of Chest Physicians

Introduction
The ACCP produces guidelines in chest medicine and

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has developed guidelines for lung cancer since 2003. The third edition of the ACCP lung cancer guidelines has been published in 2013 and is included in this review (*Table 3*) (21).

Guideline development methodology

ACCP used a formalised, systematic approach to develop the third edition of the lung cancer guidelines. A selected expert lung cancer guideline panel developed research questions in PICO [The acronym PICO refers to the 4 elements that should be included in a structured clinical question to govern systematic searches: patient, intervention, comparison and outcome. A framework commonly used in evidence-based medicine (41).] format and literature searches were designed and completed. The literature results were then screened against inclusion and exclusion criteria and formally assessed using standard quality assessment tools. If applicable, good quality meta-analysis (already published or performed by the authors specifically for the guideline) were used to inform the recommendations. Evidence summary tables and profiles were compiled for most PICO questions. Based on the evidence tables, recommendations were formulated and then graded according to the ACCP recommendation grading system. The whole guideline panel reviewed the guideline content, including formal anonymous voting to approve recommendations during face-to-face and virtual meetings. The draft guideline was then submitted through an internal and external review process before the guideline was finalised and published (42).

COI management

Each nominated guideline panel member had to submit a COI statement before the start of the guideline project. The COI statements were reviewed by the Guidelines Oversight Committee. All panellists were required to submit an updated COI statement before each meeting. COI management included strategies such as not drafting or voting on recommendations that were related to a particular conflict (42).

Funding

The majority of the guideline was funded by the ACCP. One private foundation and one pharmaceutical company financially supported the development and dissemination of the guideline. Those sponsoring companies were not allowed to participate in the guideline development process (42).

Dissemination and implementation approach

The ACCP lung guidelines are disseminated through the College's website (www.chestnet.org), the *CHEST journal* publication, National Guidelines Clearinghouse and GIN Library (42).

Planned update

The start of ongoing review is planned 1 year after publication unless the content experts, who continue to monitor the literature, suggest that recommendations need to be updated (42).

Associated resources

Additional clinical resources will be accessible in Chest Evidence. Associated patient guides will be available from www.onebreath.org (42).

American Society for Radiation Oncology (ASTRO)

Introduction

The Guidelines Subcommittee of the ASTRO identified a need for an evidence-based guideline on the use of palliative radiotherapy to lung cancer patients. The project proposal to develop this guideline was submitted and approved by the ASTRO Board of Directors in 2009 (22).

Guideline development methodology

A task force was established and assigned to review and synthesize the current available evidence to develop this guideline. The task force was divided into three topic groups and a systematic literature review was completed for each area. Evidence assessment, including the creation of evidence tables, and the formulation of the guideline content were completed and then revised by the complete expert group. The final draft was then circulated to three expert reviewers, the ASTRO legal counsel and also published on the ASTRO website for public comment. The feedback was reviewed and incorporated before the guideline was finally reviewed and approved by the ASTRO Board of Directors (22).

COI management

At the beginning of the guideline project, all members submitted COI declarations. The task group chairs reviewed all COI statements and determined that the disclosures would have no impact upon the content of the guideline manuscript (22).

Funding

Details in regards to the funding of the guidelines were not specified in the guideline document.

Dissemination and implementation approach

The guideline was formally published in the journal *Practical Radiation Oncology* (22) and the link to the article is listed on ASTRO's website.

Planned update

The ASTRO Guidelines Subcommittee will monitor this guideline and initiate an update when appropriate (22).

Associated resources

Not identified.

American Society for Clinical Oncology

Introduction

The American Society of Clinical Oncology (ASCO) has been developing clinical practice guidelines for lung cancer since 1997 and has published an update on chemotherapy for stage IV non-small lung cancer in 2011 that was eligible for inclusion in this review (23).

Guideline development methodology

The 2011 update on chemotherapy treatment for stage IV lung cancer is based on ASCO's 2009 lung cancer guideline update and addressed the clinical question, "What is the optimal duration of first-line chemotherapy for stage IV NSCLC?" from the previous guideline. The literature search for this guideline included an update of the original 2009 literature search and a systematic assessment of the updated evidence. ASCO's Guideline Procedures Manual provides details about ASCO's methodology for guideline development (24). The 2011 focused update was drafted by the co-chairs of the 2009 guideline as well as ASCO staff and was then circulated to the entire update committee for approval. The final document was reviewed and approved by ASCO's Clinical Practice Guideline Committee and Board of Directors Executive Committee. It was then submitted to *Journal of Clinical Oncology* for peer review before being finalized and published (23).

COI management

All members of the update committee completed the ASCO disclosure form prior to commencing the work on this guideline project. Further details about ASCO's COI management are published in ASCOS's COI management procedures summary (43).

Funding

Details in regards to guideline funding were not specified in the guideline publication (23).

Dissemination and implementation approach

The guideline was published in the *Journal of Clinical Oncology* (23) and is listed on ASCO's website in the clinical guideline section (*Table 3*).

Planned update

Not specified in guideline document.

Associated resources

Slide sets, patient guide and decision aids are available from http://www.asco.org/institute-quality/asco-clinical-practice-guideline-update-chemotherapy-stage-iv-non-small-cell-lung.

British Thoracic Society (BTS) and Society for Cardiothoracic Surgery (SCTS) in Great Britain and Ireland

Introduction

The BTS and the SCTS in Great Britain and Ireland had developed a guideline on the radical management of patients with lung cancer in 2001 and decided to conduct an update of this guideline to provide comprehensive guidance on selection and risk assessment of suitable patients (*Table 3*) (25).

Guideline development methodology

The guideline development group determined the guideline scope based on the previous guideline and in consultation with members from both societies. A comprehensive literature search was performed and the evidence was assessed using the Scottish Intercollegiate Guidelines Network's (SIGN) methodology. Recommendations were developed based on the evidence tables and graded according to SIGN. Research recommendations were also incorporated. The draft document was distributed amongst BTS and SCTS members and presented at society meetings for consultation and review. All feedback was assessed and reviewed by the guideline committee before the guideline was finalised, approved and published (25).

COI management

COI statements are included in the guideline publication (25).

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Funding
The BTS funded all committee meetings (25).

Dissemination and implementation approach
The guideline was published in the *Thorax journal* (25) and is also disseminated through a link on the BTS website (*Table 3*).

Planned update
2013 (44).

Associated resources
A quick reference guide is available from http://www.brit-thoracic.org.uk/Portals/0/Guidelines/Lung%20Cancer/Guidelines/LungCancerQRG.pdf.

Cancer Care Ontario

Introduction
Cancer Care Ontario, a Canadian health government agency, has published nine clinical practice guidelines for lung cancer between 2008 and 2013 (*Table 3*) covering specific clinical questions in the area of non-small cell and small cell lung cancer management (26-34).

Guideline development methodology
At Cancer Ontario, working groups consisting of two to six clinicians or content experts and one Research Coordinator were established to produce each lung cancer guideline. The working groups determined the overall guideline topic, the individual clinical questions for each topic as well as the overall scope of each lung cancer guideline. The literature review process, that formed the basis of each guideline document, consisted of two stages: first, existing lung cancer guidelines were identified to see if an existing guideline could be formally adapted. If not, a systematic review of the evidence considering the highest level evidence was conducted. After the evidence was assessed and synthesised, the working groups developed the initial recommendations. The reasoning behind each recommendation and the degree of how much it is evidence-based versus expert consensus is explicitly stated in the recommendations. All draft guideline documents went through an internal and external review process. The external review process consisted of targeted peer review and professional consultation. The draft guideline documents were then revised by the individual working groups to assess and incorporate the feedback. The process and results that arose from the consultation review

are documented in the final guideline documents. Cancer Care Ontario's guideline development methodology is described in detail in the "Program in Evidence-Based Care Handbook" published by Cancer Care Ontario (45).

COI management
Working group authors had to declare COI as soon as they started on a guideline project and provide an update when the guideline was completed. The guideline chair and research coordinator were responsible to collate the declarations and updates and manage any conflicting interest according to Cancer Care Ontario's COI policy (46). Reviewers also had to declare any competing interests.

Funding
Guideline development is supported by the Ontario Ministry of Health and Long-term Care through Cancer Care Ontario and editorially independent from its funding source (26-34).

Dissemination and implementation approach
The guidelines are published on Cancer Care Ontario's website (*Table 3*) and indexed at National Guidelines Clearinghouse and CMA Infobase. In addition, the results of several systematic reviews are published in peer-reviewed journals (47-52).

Planned update
Each year the lung cancer guidelines are assessed with a document assessment tool developed by the Program in Evidence-Based Care at Cancer Ontario to determine if any guidelines are in need of an update (45).

Associated resources
Not identified.

Cancer Council Australia (CCA)

Introduction
CCA, a not for profit cancer charity, produces evidence-based clinical practice guidelines in oncology for the Australian health care context. In 2010, CCA was commissioned by Cancer Australia (an agency of the Australian Government) to update the lung guidelines originally published in 2004. The new web-based guideline covers treatment of non-small cell and small cell lung cancer, symptom management, supportive and palliative care (35).

Guideline development methodology

A multidisciplinary working group was established and the guideline objectives and scope were defined. Clinical questions according to PICO format were developed and systematic literature searches were carried out. The literature results were screened for relevance and formally assessed. The evidence was synthesised and analysed by the assigned working group members. Each question lead author developed the initial clinical question content, including formulation of evidence statements and draft recommendations and assigning the recommendation grades according to the NHMRC grading system (53). All draft content, including the recommendations and associated grades, was then internally reviewed and approved by all members of the working party before the draft guideline was released for public consultation. All externally received comments were considered by the working party and, where necessary, changes were made to the guideline. A formal response to each comment was documented. Once the guideline was finalized, it was published on CCA's Cancer Guidelines Wiki (35). CCA's Guideline Development Handbook provides a detailed description of the applied guideline development methodology (54).

COI management

COI statements were collected from each working group member at the start of the project. The management committee had the responsibility to collect and evaluate COI statements from all nominees. All working party members are responsible to provide updated COI statements if new interests arise (35).

Funding

Co-funding to develop these guidelines was received from Cancer Australia (35).

Dissemination and implementation approach

CCA's clinical practice guidelines are available online via the CCA Cancer Guidelines Wiki (35). The link to the guidelines was distributed directly to relevant professional and other interested groups via email, print and social media campaigns as well as through meetings, national conferences and other CME events. By allowing guideline stakeholders to comment on guidelines content and submit new evidence on an ongoing basis, CCA is encouraging its stakeholders to engage with the guideline content on a long-term basis (54).

CCA is developing online learning modules to reinforce content knowledge for participants and support guideline uptake. CCA is going to pilot the development of a lung cancer QStream module originally developed by Harvard Medical School (54).

Planned update

Ongoing (54).

Associated resources

Online QStream module is in development (54).

CAP, IASLC and AMP

Introduction

Three professional societies, CAP, IASLC, and AMP, systematically reviewed the literature to develop an evidence-based guideline for selection of lung cancer patients for EGFR mutation and ALK rearrangement testing. The guideline addresses which patients and samples should be tested and when and how testing should be performed (36).

Guideline development methodology

A systematic literature review, including blinded screening for relevant studies, was performed. A formal quality assessment and data extraction was completed for all selected studies. Evidence tables were created. Based on the evidence assessment, content and evidence-based recommendations were formulated, evidence levels assigned and recommendation grades determined. In addition, recommendations based on formal expert consensus were added where appropriate and marked as such. The draft guideline then went through an extensive review process before it was finalised and published (36). The detailed methodological report is available from http://links.lww.com/JTO/A430 (55).

COI management

Before acceptance on the expert panel, all potential authors completed COI statements as per CAP' procedures and were required to disclose new conflicts at each conference call. They had to submit a general updated COI form on a yearly basis (55). The COI statements are published with the guidelines.

Funding

The guideline development was jointly funded by CAP, IASLC and AMP (36).

Dissemination and implementation approach

The guideline is disseminated through the organisations' websites and was released in *Archives of Pathology & Laboratory Medicine*, the *Journal of Thoracic Oncology*, and the *Journal of Molecular Diagnostics* (36).

Planned update

This guideline will be reviewed regularly, as mandated by publication of substantive and high-quality medical evidence that could potentially alter the original guideline recommendations (36).

Associated resources

A summary of recommendations is available from http://www.cap.org/apps/docs/membership/cap_iaslc_amp_summary_of_recommendations.pdf. A patient guide is available from http://www.cap.org/apps/docs/membership/lc_patient_guide.pdf. A frequently asked question sheet is available from http://www.cap.org/apps/docs/membership/lc_faqs.pdf.

National Institute for Health and Care Excellence

Introduction

NICE is a UK-based health authority that provides national guidance and advice to improve health and social care. In 2011, NICE published a revision of the clinical practice guideline for lung cancer titled "Lung cancer. The diagnosis and treatment of lung cancer" (37).

Guideline development methodology

The methods that were used to develop the lung cancer guideline are in accordance with those set out by NICE in *The guidelines manual* (56). After the decision was made to update the lung cancer guideline, the guideline scope was defined and a lung cancer guideline development group was established. The group formulated clinical questions using the PICO framework where applicable. Comprehensive, systematic literature searches were carried out for each question and the evidence critically appraised and assessed. Health economic evidence was also included, assessed and synthesized. Based on the evidence synthesis, recommendations were developed and agreed upon by the working group. Qualifying statements about the strength of evidence, about the benefits and harms for the intervention being considered, the degree of consensus within the GDG and the costs and cost-effectiveness of an intervention were added. The guideline draft went through a consultation

process, which was documented and published as a separate report on the NICE website. Based on the stakeholder comments, the guideline content was revised and went through a pre-publication check process, before the final guideline version was published (37).

COI management

At the start of the guideline development process, all COI statements from each guideline development group member were recorded. At each subsequent meeting, members declared any new, arising interests. For group members, that declared any conflicting interests, an evaluation took place and a management plan was implemented (37). The code of practice for declaring and dealing with conflicts of interest outlines the COI management procedures in further detail (57).

Funding

NICE commissioned the National Collaborating Centre for Cancer to develop this guideline. The health economic analysis was conducted by the London School of Hygiene and Tropical Medicine and funded by the National Collaborating Centre for Cancer (37).

Dissemination and implementation approach

This guideline is disseminated as web-based and short and long PDF versions on the NICE website. Numerous implementation tools have been developed to facilitate guideline update (see under associated resources). The NICE guidelines manual outlines the guideline dissemination and implementation approach for NICE guidelines in detail (56).

Planned update

After three years, the guideline will be formally evaluated to assess if an update is required (37).

Associated resources

A short version of this guideline, containing the key priorities, key research recommendations and all other recommendations, and a Quick Reference Guide (QRG) are available from http://www.nice.org.uk/guidance/index.jsp?action=byID&o=13465.

The following implementation tools are available from http://www.nice.org.uk/guidance/index.jsp?action=byID&o=13465: baseline assessment tool, clinical audit tool, costing report, costing template, multiple guidance audit tool, slide set, online educational tool about referral in case of suspected lung cancer.

Discussion

Considerable resources have been spent internationally on the development of lung cancer guidelines. This review article highlights that health professionals specialising in the treatment of lung cancer, patients and other stakeholders have access to numerous clinical practice guidelines developed for different local contexts. As the major concern around clinical practice guidelines is around quality, especially rigour of development, validity of recommendations and editorial independence, guideline users are encouraged to formally assess the quality of any identified lung cancer guideline (58). The guideline quality assessment instrument Agree II provides a validated tool to complete such quality assessments (59).

It was not part of this review to analyse and compare recommendations across guidelines addressing the same areas, nevertheless we are aware that variation does exist. For example, in patients with stage I non-small cell lung cancer who cannot tolerate surgery, the ACCP recommends stereotactic body radiotherapy (SBRT) (21), whereas NICE recommends patients should be offered continuous hyperfractionated accelerated radiotherapy (CHART) (37). ACCP does not mention CHART at all (21); NICE offers no guideline on SBRT, but recommends that further research should be undertaken (37). Undertaking a detailed content comparison across the identified lung cancer guidelines and investigating any variations, may be a worthwhile project to emerge from this initial review. It would be of interest to know if the reasons for any variations are resource related (for example Alberta Health Services does not recommend CHART because it is unavailable there), or a result of regional/cultural preferences in practice (for example the level of therapeutic aggression or nihilism). An example of the latter is the ACCP guideline for patients who have undergone resection of an isolated brain or adrenal metastasis, that adjuvant chemotherapy is suggested (21), whereas NICE only recommends adjuvant chemotherapy for patients without metastatic disease (37). The international variation in cultural attitudes to what are reasonable levels of medical intervention (as suggested by this example) could present an obstacle to the ultimate development of truly universal guidelines.

Compiling an overview of available lung cancer guidelines also pinpoints general challenges in the area of guideline development. Lung cancer guidelines, that follow an international standard, are presented in a validated, uniform format and are published together with the results of independently performed quality assessments, are still a vision of the future, even though significant efforts have been made to provide standards, methodologies and presentation guidelines (1,60-62).

Successful dissemination and implementation of lung cancer guidelines is another challenging area (39,63,64). Even if high quality evidence-based guidelines are available, it does not guarantee successful uptake by health professionals. Guideline developers, health care organisations, and governments need to put adequate resources into guideline dissemination and implementation and follow multiple implementation strategies to maximise uptake (39). Further there are many competing sources of information on lung cancer management besides guidelines which are readily available to health professionals and consumers. Although they may lack the endorsement of respected learned societies, these other sources, usually web-based, having avoided a lengthy development process, may provide more up-to-date information than traditional guidelines, and so become the first port of call for the information seeker. Conversely, without the rigour under which the guidelines are produced, use of that approach might lead to acceptance of faulty information.

It is therefore critical to keep the guidelines current if they are to be relevant and well used. Collaborating on lung cancer guidelines internationally by sharing literature searches and assessments is considered an effective approach to reduce duplication of effort and help developers keep the existing guidelines current (65). We hope this review provides an information starting point to bring together potential future collaborators with a view to developing integrated, dynamic, so called "living guidelines", which can then be adapted to suit the different cultural and organisational contexts.

Summary

The aim of this review article was to provide a comprehensive overview of available clinical practice guidelines in the areas of small cell and non-small cell lung cancer. 22 clinical practice guidelines produced by 12 organisations with varying scopes and developed for different regions were identified and key features summarised. Health professionals in the area of lung cancer have no shortage of guidelines to assist the clinical decision making process. Future research needs to focus more on dissemination, implementation, guideline adherence and their effect on disease outcome. It is hoped this article will

be a useful resource for clinicians and other stakeholders to easily access these different guidelines and assess relevance to their own practice. We also hope it may lead to organisations to pool their resources to develop consistent, internationally relevant guidelines for what is, after all, a global disease.

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Screening for lung cancer with low-dose computed tomography: a review of current status

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Abstract: Screening using low-dose computed tomography (CT) represents an exciting new development in the struggle to improve outcomes for people with lung cancer. Randomised controlled evidence demonstrating a 20% relative lung cancer mortality benefit has led to endorsement of screening by several expert bodies in the US and funding by healthcare providers. Despite this pivotal result, many questions remain regarding technical and logistical aspects of screening, cost-effectiveness and generalizability to other settings. This review discusses the rationale behind screening, the results of on-going trials, potential harms of screening and current knowledge gaps.

Keywords: Lung neoplasms/mortality; mass screening tomography; helical computed; early detection of cancer/methods

Introduction

The rationale for lung cancer screening

Lung cancer caused an estimated 1.4 million deaths in 2008 (1), and is the leading cause of cancer death worldwide. Incidence and mortality closely follow smoking trends with a time-lag of twenty years. This explains why death rates are falling or plateauing in countries such as the US, yet rising in others such as China (2,3). Lung cancer carries a poor prognosis with reported overall five year survival between 8 and 16% in Europe and the USA, and between 6% and 32% in China (4-6).

Currently 25-30% of patients present with localised, potentially curable disease. Five year survival for those with pathological stage IA non-small cell lung cancer (NSCLC) is 73% whereas metastatic disease has a dismal prognosis (13% 5-year survival) (7,8).

Given that lung cancer has a detectable pre-clinical phase, effective treatment, especially surgery, with effective and potentially cost-effective applicable screening methods, it would seem to fulfil the criteria for screening first described by Wilson and Jungner (9) (*Box 1*). Although early screening studies using plain chest radiography (CXR) had methodological drawbacks (11), it is generally accepted that CXR screening does not confer a mortality benefit, a conclusion reinforced by the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (12). In contrast, computed tomography (CT) is a far more sensitive imaging modality which has been studied for its potential utility in lung cancer screening over the past 25 years. Recently, the National Lung Screening Trial (13) showed that low-dose CT (LDCT) screening reduced lung cancer mortality by 20% compared with CXR screening. This was the first demonstration in a randomized clinical trial of a mortality reduction with screening. In response to these findings several expert bodies in the USA issued guidelines for screening high-risk populations and the US Preventive Services Task Force has awarded a Grade B draft recommendation (14-17).

Condition
• The condition should be an important health problem.
• There should be a recognisable latent or early symptomatic stage.
• The natural history of the condition, including development from latent to declared disease should be adequately understood.
Test
• There should be a suitable test or examination.
• The test should be acceptable to the population.
Treatment
• There should be an accepted treatment for patients with recognised disease.
Screening program
• There should be an agreed policy on whom to treat as patients.
• Facilities for diagnosis and treatment should be available.
• The cost of case-findings (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
• Case-findings should be a continuing process and not a 'once and for all' project.

Box 1 Principles of early disease detection [adapted from Wilson and Jungner (9)].
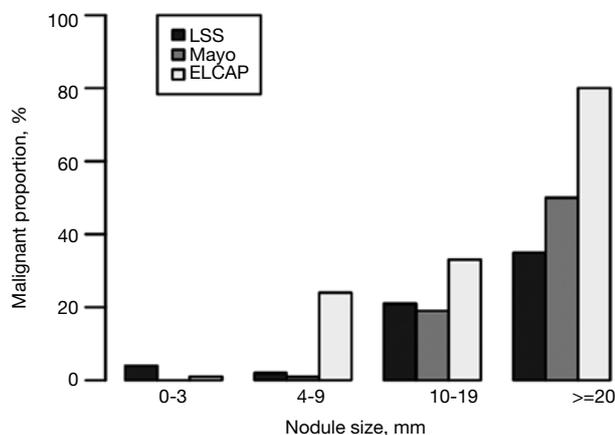
Figure 1 Nodule size correlates to risk of malignancy*. LSS, Lung Screening Study; Mayo, Mayo Clinic Study; ELCAP, Early Lung Cancer Action Project. *Cut-off sizes were slightly different between studies (29-31).

LDCT screening--practical issues and technical considerations

One of the most important issues confronting those who wish to consider implementation of LDCT screening in high-risk populations is the problem of the high rate of positive examinations, primarily pulmonary nodules.

Nodule detection

Pulmonary nodules can be defined as rounded or irregular opacities, well or poorly defined, measuring up to 3 cm in diameter (18). There is inherent subjectivity in identifying nodules, reflected in inter- and intra-reader variability, even amongst experienced radiologists (19,20).

A considerable proportion of nodules may be missed at first reading and identified retrospectively at later scans (21). Nodule detection may be increased by using a second reader (22), image formatting, e.g., to maximum intensity projections (MIPs) (23-25) or by using computer aided detection (CAD) software as a "second reader" (26-28).

Nodule assessment

Nodules are best classified in four important ways: size, attenuation, presence/absence of calcification and, once a follow-up scan has been obtained, interval growth rate.

Size

Nodule size is the most important predictor for malignancy (*Figure 1*) (29-31). Detailed analysis of baseline NLST results found the positive predictive values (PPV) for malignancy increased significantly from 1.7% for nodules 7-10 mm in diameter to 11.9%, 29.7% and 41.3% for those 11-20, 21-30 and >30 mm diameter respectively (32). However even very small nodules (micronodules) have some risk of malignancy, e.g., 3 of 230 nodules <5 mm diameter (1.3%) at baseline scan followed for one year (33).

Attenuation

Certain calcification patterns and intra-nodular fat reliably
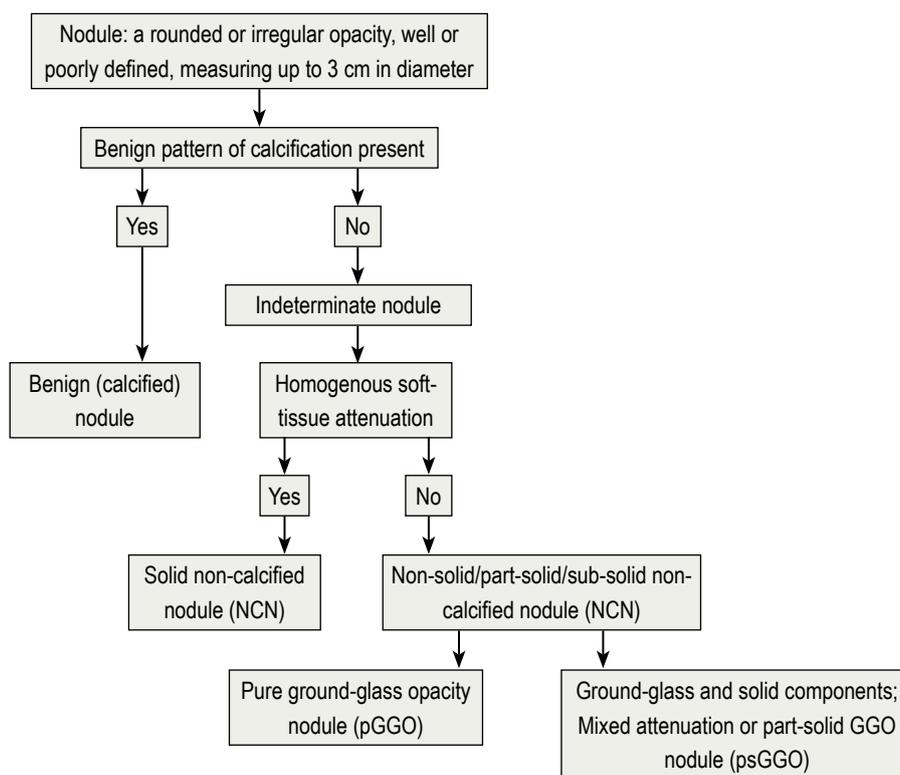
Figure 2 Classification of nodules detected by LDCT screening.

indicate benignity (34), however, many nodules are too small to resolve internal features and are simply classified as 'non-calcified' nodules (NCNs). NCNs are common and detected in 25-50% of LDCT scans.

The majority of NCNs are of 'solid' (soft-tissue) radiological attenuation. The remainder are classified as non-solid nodules (NSNs) and subdivided into pure ground-glass (pGGO) or mixed (part-solid) attenuation nodules (solid and ground-glass components; psGGO). Synonyms vary between studies (*Figure 2*). The significance of GGOs is contentious as discussed below.

Ground glass opacities

The ELCAP study reported positive findings in 233/1,000 baseline scans. 19% of lesions were pGGO or psGGO (prevalence 4.4%; slice thickness 10 mm). Twenty-seven cancers were detected. After adjusting for size, the malignancy rate was 63% for psGGO, 32% for solid nodules and 13% for pGGOs (35). Other studies highlight the importance of a new or increasing solid component within NSNs, a finding highly suggestive of lung cancer (36-38). More recent studies demonstrate many NSNs spontaneously resolve. Felix (39)

reported 75 GGOs in 37/280 patients (prevalence 13%; slice thickness 0.75 mm). The population was atypical for screening studies as over half had a history of lung or head and neck cancer. Approximately half the GGOs were present at baseline and half disappeared over a median 29 months follow-up. No morphological features allowed reliable discrimination between resolving and non-resolving GGOs. Kwon (40) reported 69 pGGO and 117 psGGO mostly detected by screening in 186 patients (total screenees not reported; slice thickness 5 mm). After 3 months, 45% regressed or disappeared. Malignant and benign lesions were similar in size (average 15-16 mm). Only 27% (33/122) were malignant but this may reflect a short follow-up time (mean 8.6 months; 64 lesions were still under active follow-up at publication). A second Korean study (41) identified 126 NSNs >5 mm diameter in 93 of 16,777 (0.5%) asymptomatic screenees. Forty-four had never smoked. 70% of NSNs were transient. Younger age, detection at a follow-up scan, blood eosinophilia, multiple lesions, larger solid component and ill-defined border independently predicted transiency. Mario (42) reported 76 NSNs retrospectively identified in 56/1,866 baseline screening scans in a high-

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risk screening cohort (prevalence 3%; slice thickness 0.75 mm) and followed for 50±7.3 months. Only 13 nodules were prospectively identified. 40 of 48 pGGOs (83%) resolved, decreased in size or remained stable. 16 of 28 psGGOs (57%) resolved or remained stable. Overall, 74% NSNs resolved, decreased in size or remained stable and 26% progressed. One psGGO (2%) was confirmed as lung adenocarcinoma.

In summary, perhaps as many as 50-70% of NSNs detected on modern thin-slice CT scans are transient but predicting which will persist is currently beyond our ability. The data suggest that a substantial difference in NSN prevalence between Western and Asian populations is unlikely. In view of slower growth rates for non-solid tumours (37,43) active surveillance for >2 years may be prudent for non-resolving NSNs (44).

Growth rate

Once a follow-up scan is obtained, assessment of growth can be made. Generally, absence of growth in a solid nodule over a 2 year period makes malignancy unlikely (45), although a contemporary review found the underpinning data (based on CXR studies from the 1950s) less than compelling (46).

Growth is best assessed by CT. For example, assuming exponential growth, a 5 mm diameter nodule with a volume doubling time (VDT) of 460 days will only increase to 6 mm diameter after one year and 7.2 mm after two years-changes which may not be measurable on CXR but which can be appreciated on CT. However, reproducible measurement is difficult: the 95% confidence intervals (CIs) for inter-reader measurements of nodules with a mean diameter of 8.5 mm were +/− 1.73 mm in one study (47). Semi-automated volumetric measurement using computer software may be more reproducible and accurate (48,49) and is the basis of nodule management in the NELSON trial (47-50). However even this is subject to error, e.g., with smaller nodules, in the presence of motion artefact (51), nodules attached to other structures and NSNs (52).

There are limited long-term data supporting the two year stability guideline for sub-centimetre NCNs; In an Irish study (53) 83 subjects with NCNs <10 mm stable over two years were imaged again at seven years. Virtually all nodules remained unchanged at the seven-year CT, however one 3 mm GGO grew to 15 mm in four years and was subsequently diagnosed as (what was previously called) bronchioloalveolar cell carcinoma. Thus ideally, the two year stability guideline suggested by CXR studies should be

validated in larger, contemporary CT datasets.

The importance of baseline size and interval growth is shown in data from the NELSON study (54). 891 solid nodules 5-10 mm diameter were followed for one year. 743 nodules, all with smooth margins and/or attached to fissures, pleura, or vessels (contact length ≥50% of nodule diameter) were benign and excluded from multivariate analysis. Spiculated, irregular or lobulated nodules were analysed further. 10 of 69 (14.5%) nodules with spiculated or irregular margins and 6 of 168 (3.6%) nodules with lobulated margins were malignant. At baseline the only characteristic that predicted malignancy was volume ≥130 mm^3 (OR 6.3; 95% CI: 1.7 to 23.0). At 3-months, baseline volume and VDT <400 days were significant (OR 4.9; 95% CI: 1.2 to 20.1 and OR 15.6; 95% CI: 4.5 to 53.5, respectively); At one year only VDT was predictive (OR 213.3; 95% CI: 18.7 to 2,430.9). Very few nodules showed change in margin or shape over 12 months, so these features were unable to distinguish malignant from benign nodules (55).

Other morphological features

Diederich (56), in a study of 133 consecutive resolving nodules, found the demographic and morphologic features of resolving and non-resolving nodules overlapped so greatly that none could be used to predict outcome over two years' follow-up.

Features of benignity noted by Takashima after two years follow-up (72 nodules ≤10 mm diameter including 25 cancers) were polygonal shape, subpleural location, solid attenuation and elongation (higher long-axis-to-short-axis diameter ratio) (57). Long-term analysis of 234 similar nodules (perifissural with any of the following features: polygonal shape, long-axis-to-short-axis diameter ratio >1.78, peripheral location, vascular attachment) detected in 98/146 consecutive screenees found the nodules were multiple in half the subjects, ranged from 1-13 mm diameter, were mostly triangular or oval (86%), inferior to the carina (84%) and had a septal connection (73%) (58). 139 screenees were accounted for after 7.5 years, and none of the perifissural nodules had developed into cancers. These types of nodules most probably represent intrapulmonary lymph nodes, however histopathologic confirmation was not performed in either study (57,58).

The difficulty in predicting which nodules might be malignant is highlighted by low PPV in screening studies; with a cancer prevalence of 1-2% the PPV of a nodule designated by the radiologist as 'suspicious' or large in size or with VDT <400 days actually being malignant was only

Table 1 Comparison of nodule management protocols for three leading LDCT studies.

	Nodule characteristics (attenuation, diameter, volume)		Recommended action	Interval findings	Recommended action
Small	NLST	<4 mm d_{max}	12 m LDCT		
	NEL	<50 mm^3 without benign characteristics			
	IE	Solid/ part-solid <5 mm d_{mean}; non-solid: any size			
Intermediate			3-6 m LDCT (may vary up to 24 m according to level of suspicion)	No growth[†]	→ 12 m LDCT
				Growth <7 mm	→ 3-6 m LDCT or refer to pulmonologist
	NLST	Solid 4-10 mm d_{max}		Growth ≥7 mm	→ Refer to pulmonologist
		Pure GGO 4-10 mm d_{max}	6-12 m LDCT	As per solid 4-10 mm nodules	
	NEL	Solid: 50-500 mm^3; Solid, pleural based: 5-10 mm Mixed: GGO component: ≥8 mm d_{mean} or solid component: 50-500 mm^3 Pure GGO: ≥8 mm d_{mean}	3 m LDCT	Growth[††]	→ Refer to pulmonologist
	IE	Solid/ part-solid 5-15 mm d_{mean}	3 m LDCT (preferred option) or Antibiotics & 3 m LDCT if infection possible or	Growth[†††]	→ Biopsy
			PET scan if solid/solid component >10 mm	PET scan negative	→ 3 m LDCT
Large	NLST	Solid >10 mm d_{max}	Refer to pulmonologist		
		Other suspicious finding			
	NEL	Solid: >500 mm^3; Solid, pleural based: >10 mm Mixed, solid component: >500 mm^3			
	IE	Solid/ Mixed >15 mm d_{mean}			

Key: *NLST-NLST, NEL-NELSON, IE-I-ELCAP*; m, month; d_{mean}, mean of maximal diameter and width viewed on same CT slice; d_{max}, maximal diameter on axial CT slice; PET, Positron-emission tomography; GGO, ground glass opacity attenuation nodule; Definitions of growth minimum significant change: [†], >10% increase in diameter; [††], ≥25% increase in volume after at least a 3 months interval; [†††], Minimum change in nodule diameter/solid component of part-solid nodules to define significant growth: for nodules <5 mm in diameter, ≥50%; for nodules 5-9 mm in diameter ≥30%; for nodules >10 mm in diameter ≥20%. Adapted from NLST (60) NELSON (52), I-ELCAP (61).

around 35% in two studies (50,59).

Nodule management protocols
LDCT nodule management protocols reflect the association of size and growth with malignancy. The protocols from the three largest studies, NLST, NELSON and I-ELCAP are summarized in *Table 1* (52,60,61). These protocols have been applied to 26,722, 7,557 and 31,567 LDCT screenees respectively although I-ELCAP has no control arm. Size category definitions vary slightly, but in general terms 'micronodules' (usually less than 4-5 mm diameter) are followed after 12 months, large

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nodules (>10-15 mm diameter) are sent for immediate investigation and medium size nodules are followed-up to determine growth. Most studies use linear measurements of nodule size but the NELSON study uses volumetric measurement (50). Retrospective analysis of I-ELCAP data suggested the threshold to define a 'positive' baseline scan may be too inclusive; increasing the threshold to 7-8 mm (mean of maximal diameter and width) may reduce the false positive rate and subsequent work-up by 50-68% but at the cost of diagnostic delay for 5-6% of true positive cases (62). To date, only the NLST protocol has been proven to reduce lung cancer mortality.

Non-nodule (incidental) findings (IFs)

Non-cancer IFs such as coronary artery calcification (CAC), emphysema, and thyroid nodules are common but rates vary widely depending on study definitions and recording protocols. A NELSON substudy (n=1,929) found an IF rate of 81%. Six percent of participants received follow-up but only 1% had clinically important findings arguing against systematically searching for IFs (63). A Canadian study (n=4,073) found IFs in 19%; Approximately half would have required follow-up and 0.8% immediate action (64).

LDCT screening may be an opportunity to screen for other conditions which can be detected on chest CT such as CAC, chronic obstructive pulmonary disease (COPD) and osteoporosis (65,66). This may increase cost-effectiveness and provide better global outcomes but is currently untested. Radiologist-detected emphysema on CT scans appears to confer an independent increased risk of lung cancer (OR 2.1) (67) and may have the potential to help determine screening frequency following baseline scan (68) (i.e., more frequent screening for those with visually-detected emphysema), but this hypothesis remains to be tested.

CAC, a risk marker for cardiac events (69) is potentially the most important IF. Worldwide, smoking is estimated to cause 0.8 million deaths from acute heart attacks annually (70). The ELCAP investigators found varying degrees of CAC in 64% of 4,250 screenees (71). They developed a simple visual scoring system which was able to stratify cardiovascular death risk in a second cohort of 8,782 screenees followed for a median of six years (72). The NELSON study reported higher hazard ratios for all-cause mortality with increasing CAC in 958 participants followed for 21 months (73). However these findings do not appear to be reflected in NLST data where approximately 75% of all deaths were from non-

lung cancer causes (13). Cardiovascular illness accounted for 486/1,865 (26.1%) deaths in the LDCT group and 470/1,991 (23.6%) in the CXR group. The 6.7% reduction in all-cause mortality in the LDCT group lost statistical significance when lung cancer deaths were removed from the comparison (3.2%, P=0.28) indicating that reduced lung cancer mortality was largely responsible for the reduction in all-cause mortality (13). Clinically significant IFs were identified in 7.5% of all scans and although details of CAC prevalence and follow-up are not yet reported, it seems unlikely that identification of CAC on LDCT screening made a significant impact on cardiovascular mortality in this study.

Thus IFs are common but mostly of little significance. Exhaustive investigation of IFs will increase the costs of screening through downstream investigation and follow-up, and should be accounted for in cost analyses. Further analysis of CAC and possibly other conditions in screening studies is warranted.

Screening by LDCT-effectiveness

Observational studies

The earliest LDCT screening studies were observational cohort studies from the USA and Japan (*Table 2*). CT appeared to be 3-4 times more sensitive than CXR in the ELCAP study, and the majority of tumours were stage I. Entry criteria were varied. Studies recruiting younger participants (<50 years old) and never-smokers had lower prevalence and/or incidence rates. For example, in a Japanese study (75) in which the majority of screenees had never smoked, cancer prevalence was only 0.4% compared to ELCAP 2.7% (31). These results underline the importance of recruiting a high-risk population. Subsequently, most studies follow the ELCAP strategy recruiting older persons with extensive smoking histories. Risk stratification is an area of current research interest and is discussed later.

Although very promising, these studies lacked control groups to allow estimation of mortality benefit. Survival, as a surrogate endpoint of effectiveness, is subject to several biases and cannot therefore be used to prove screening efficacy (*Box 2*). To add to the debate, studies modelling mortality benefit markedly diverged in their conclusions (78-80).

Randomised controlled trials

The randomised control trials of LDCT screening are

Table 2 Results from selected observational LDCT lung cancer screening studies

	Year	n	Cohort characteristics	Additional tests	Cancer prevalence %	Cancer incidence %	Stage I tumours %	5-year survival
ELCAP (31)	1992	1,000	>60 yr old; >10 PY smoking	CXR	2.7	0.6	85	65%
ALCA (74)	1993-95	1,611	40-75 yr old; 14% non-smokers; 16% <50 yr old	Sputum cytology, CXR	0.87	0.28	82	70%
Matsumoto Research Centre (75)	1996-98	5,480	40-74 yr old; 54% never smokers; 10% <50 yr old	Sputum cytology	0.41	0.23-0.56	83	83%
Mayo Clinic (76)	1999	1,520	>50 yr old; >20 PY smoking	–	1.9	2	56	–
I-ELCAP (77)	1993-2005	31,560	>40 yr old; 16% never smokers	–	1.3	0.3	85	80% (10 yr)

Bias in screening studies
- This box describes the three most important survival biases in screening studies. Survival cannot be used as a robust endpoint as, without a control group, there is no way of determining the relative contribution of each bias. Relative mortality between the intervention (screened) and control group is the best endpoint to use.

Lead-time bias
- Survival is measured from time of diagnosis to time of death. CT is more sensitive than CXR and will therefore detect smaller tumours earlier. Even though there may be no benefit in terms of reducing mortality, survival will appear longer for CT detected tumours as the diagnosis was simply made earlier.

Length bias
- Screening tends to detect slower-growing tumours and miss more aggressive ones. Rapidly-growing and aggressive tumours are more likely to grow and metastasise in the between-scan interval, and thus be missed whereas slowly growing tumours have a longer preclinical phase and are more likely to be detected by screening. As screening selects for less aggressive tumours, outcomes are more favourable thus survival may appear better in the screened group.

Overdiagnosis bias (pseudodisease)
- The detection of tumours which are never destined to cause morbidity; the patient dies from competing causes 'with' the cancer rather than 'from' it. In the absence of screening the cancer would never have been diagnosed in the lifetime of the person. Most of these tumours will be slow-growing or indolent. People at risk of lung cancer have a high risk of dying from other causes because of the shared risk factors of smoking and older age. Overdiagnosis bias makes screening appear to be more successful than it really is but essentially has detected non-lethal disease. This is a major problem in prostate cancer screening where, for example, as many as 60% of cases detected by prostate-specific antigen screening may be overdiagnosed (10). Individuals with overdiagnosed cancer undergo investigation and treatment with no hope of living longer. This futile management exposes patients to unnecessary harms and diverts finite health resources from other areas. Overdiagnosis in lung cancer screening has yet to be quantified (see text).

Box 2 Survival bias in screening studies.

summarized in *Table 3*. Two trials, the NLST (USA) and NELSON (Holland/Belgium), have adequate statistical power to detect a reduction in lung cancer mortality. The smaller European studies are planning a meta-analysis (93). All European studies (except for Depiscan and DANTE) randomised LDCT screening against no screening, the current standard of care.

The most important RCT result to date is from the NLST study (13). This landmark study randomised 53,454 high risk volunteers to three rounds of screening by CXR or LDCT (baseline, year 1 and year 2) and followed up for a median of 6.5 years. Eligibility criteria included: current or former smokers with ≥30 pack year smoking history (quit no more than 15 years previously); No history of lung or

Table 3 Randomized clinical trials of lung cancer screening using low-dose computed tomography; Target group and screening schedule

Study	Age range	Smoking history	Participants (baseline), n			Screening schedule (years)**	Control group	Total period of follow up	Years of recruitment	Completion/ expected completion
			LDCT arm	Control arm	Total					
NLST, USA (13,81)	55-74	Current or ex-smokers >30 PY, quit <15 yr	26,722	26,732	53,454	0,1,2	CXR	5	2002-4	2009
LSS, USA (pilot study) (82)	55-74	Current or ex-smokers >30 PY, quit <10 yr	1,660	1,658	3,318	0	CXR	1	2000	2001
DANTE, Italy (83)	60-74 (men only)	Current or ex-smokers >20 PY	1,276	1,196	2,472	0,1,2,3,4	Annual clinic review†	4	2001-6	2010
Dépiscan, France (pilot study) (84)	50-75	Current or ex-smokers >15 PY, quit <15 yr	336	285	765	0,1,2,3	CXR	2	2002-4	2004
NELSON, The Netherlands and Belgium (50,85,86)	50-74	Current or ex-smokers >15 PY, quit <10 yr	7,915	7,907	15,822	0,1,3	Usual care (no intervention)	10	2003-6	2015
DLCST, Denmark (87)	50-70	Current or ex-smokers >20 PY, quit <10 yr	2,052	2,052	4,104	0,1,2,3,4	Usual care (no intervention)	10	2004-6	2014
ITALUNG, Italy (88)	55-69	Current or ex-smokers >20 PY, quit <10 yr	1,613	1,593	3,206	0,1,2,3	Usual care (no intervention)	4	2004-6	–
MILD, Italy (89)	49-75	Current or ex-smokers >20 PY, quit <10yr	1,190 annual; 1,186 biennial	1,723	4,099	Annual or biennial for 10 years	Usual care (no intervention)	10	2005-onwards	Ongoing-started 2005
LUSI, Germany (90)	50-69	Current or ex-smokers >15 PY, quit <10yr	2,029	2,023	4,052	0,1,2,3,4	Usual care (no intervention)	5	2007-onwards	Ongoing-started 2007
UKLS (pilot study), United Kingdom (91,92)	50-75	5% risk of developing lung cancer in 5 years (Liverpool Lung Project risk model)	2,000*	2,000*	4,000	0	Usual care (no intervention)	10	2011-onwards	Ongoing-started 2011

*Planned recruitment; PY, Pack years (cigarettes per day/20× duration of smoking in years); CXR, chest radiograph; **Screening schedule indicates which year the scans are performed with '0' indicating baseline scan; †, all participants received CXR + sputum cytology at baseline.

Table 4 Factors affecting screening cost-effectiveness

Population	Screening intervention	Nodule management	Clinical
Disease prevalence in the target population (determined by risk, e.g., age, smoking history)	True-positive rate	Definition and rate of 'positive' scan results	Stage distribution of detected disease
Uptake of screening	False-positive rate	Nodule follow-up algorithm	Treatment costs
Adherence to screening	Over-diagnosis rate	Invasive diagnostic procedure rate	Investigation and treatment of incidental findings
	Screening frequency (interval between scans)	Adverse event rate	
	Screening duration (years)	Cost of diagnostic work-up	
	Lung cancer mortality reduction		
	Effectiveness of smoking cessation program		
	Radiation exposure		
	Cost of screening scan		

other cancer in the past five years; No current symptoms suggesting lung cancer; No chest CT in the previous 18 months. The study demonstrated a relative reduction in lung cancer-specific mortality of 20.0% in the LDCT arm (95% CI: 6.8 to 26.7; P=0.004).

Despite this positive result, several issues remain particularly generalizability and cost-effectiveness. The NLST authors stated their data alone are 'insufficient' to fully inform lung-cancer screening recommendations (13) and the Position Statement from the International Association for the Study of Lung Cancer (IASLC) Task Force on CT Screening reminds us that screening benefit, costs and potential harms must be defined in a 'cultural context', i.e., positive results seen in USA studies may not translate directly to other countries or healthcare systems (94). Additionally, the negative effects of screening and knowledge gaps, discussed below, must be considered.

Screening adherence

Good adherence is important to the success of mass screening. NLST reported 95% adherence across all three screening scans and NELSON reported 97% at year two. Long-term observational studies report 80% adherence at year five and 86% at year seven (76, 95). How this will translate to the 'real world' is not known.

Downstream healthcare use

Positive scans and incidental findings require clinical and radiological follow-up. Healthcare use may rise in the first six months following screening but return to baseline levels 6-12 months after screening and appears independent of result (i.e., negative, indeterminate or suspicious findings) (96). Although this study found doctor visits increased by 50%, in absolute terms this only meant one extra visit per participant (96).

Cost-effectiveness

Cost-effectiveness, a fundamental requirement of screening implementation, remains to be addressed. It depends on a complex mix of factors which vary from program to program and country to country (Table 4). Estimates vary widely depending on the underlying assumptions and models used, making conclusions difficult to draw (97). Using NLST data, Goulart estimated that if 75% of the eligible US population underwent screening, the cost to avoid one lung cancer death would be $240,000 (98). McMahon's analysis paid particular attention to a model combining screening and smoking-cessation (99). The estimated cost per Quality Adjusted Life Year (QALY) in a cohort of 50 years old could be below $75,000/QALY if quit rates could be doubled from

the background rate. From a health insurance perspective cost estimates were highly favourable (100); screening high-risk 50-64 years old would cost $1 per insured member per month, and the cost per life-year saved would be below $19,000.

To date, heterogeneous modelling methodologies and underlying assumptions have led to highly conflicting cost-effectiveness estimates. The final analysis from NLST has yet to be reported in a peer-reviewed format and is eagerly awaited. Preliminary data (101) suggest that it will be cost-effective with an Incremental Cost Effectiveness Ratio (ICER) of $72,900 US per QALY.

Negative effects of screening

Screening for any disease has risks and benefits. The balance helps determine overall effectiveness and acceptability of the screening program. The main negative effects are discussed below.

Radiation

It is generally accepted that ionising radiation is a cause of cancer without a lower "dose" threshold, although the absolute level of risk is debated (102,103). Minimising radiation dose according to the ALARA principle ('as low as reasonably acceptable') (104) is particularly important when screening asymptomatic, healthy subjects. CT radiation dose is determined by many factors including tube current, tube voltage, the use of filters and scan length (Z-axis). In screening studies, the most common way to limit dose is to adjust tube current (milliamperes, mA) (105) according to patient weight. This can degrade image quality as image noise (grainy mottling) is inversely proportional to the square root of the radiation dose. Fortunately the inherently high contrast between air-filled lung parenchyma and soft tissue lesions means pulmonary nodules are well-visualised. The mean effective dose from screening CT scans can be reduced from 8 mSv (standard CT chest) to approximately 1.5 mSv without significant deterioration in resolution or image quality (13,106,107). Although the lower radiation dose results in more noise it has been shown to provide adequate diagnostic pictures and is thus the current standard for screening (108-110). Total radiation dose can be further limited by restricting the scope of follow-up CTs to a region of interest surrounding the nodule(s) in question rather than covering the entire chest, so-called 'limited' LDCT (111).

Smoking appears to interact synergistically with ionising radiation. In absolute terms the risk of cancer from LDCT is small, perhaps only an excess lifetime risk of 0.85% (95% CI: 0.28% to 2.2%) for the worst case scenario of a 50-year-old female smoker receiving 25 annual LDCT scans. This compares to a 17% risk of developing lung cancer (112). Berrington de Gonzalez estimated the cumulative risk of excess death from lung cancer from LDCT screening in 50-year-old smokers to be 2 per 10,000 men screened and 5 per 10,000 women screened. Additionally an estimated 3 cases of breast cancer per 10,000 women screened may occur (113). The NLST estimated the number needed to screen (NNS) to prevent one death from lung cancer was 320, equating to a rate of 30 fewer deaths per 10,000 screenees (13) a larger benefit than the radiation harm particularly as the cancers induced occur after a delay of many years and the lives saved are over the short term. Estimates from the ITALUNG RCT reached similar conclusions with an estimated 1.1 excess deaths per 10,000 screenees compared to approximately 15-100 lives saved per 10,000 screenees (women and men respectively) assuming a 20% mortality reduction from screening (114). Thus the radiation risk-benefit ratio of LDCT screening appears quite favourable in older populations of smokers.

Adverse events

Adverse events may result from investigation of LDCT findings. As 25-50% of screenees may have one or more nodules detected, a potentially large reservoir of patients at risk exists. In the NLST the cumulative chance of a positive screening scan was 39.1%.

Despite guidelines (115), significant variation in pulmonary nodule biopsy rates (14.7 to 36.2 per 100,000 adults) and complication rates have been found between hospitals in the USA (116). The risk of haemorrhage and pneumothorax requiring intercostal catheter drainage (ICC) were 1.0% and 6.6% respectively. Complications were associated with an increased length of stay and risk of respiratory failure. Those at highest risk were smokers, persons aged 60 to 69 years, and those with COPD, i.e., the types of patients targeted for screening. LDCT screening study adverse event rates may be slightly higher than the above study but this probably reflects more rigorous, prospective reporting. There appears to be no standard way of defining or reporting adverse event data which makes some studies difficult to compare directly. 'Number

of events per 10,000 scans' may be a useful metric to allow cross-study comparison.

A study of 4,782 participants (117) screened using the I-ELCAP protocol reported a biopsy rate of 2.6% (n=127) including 110 percutaneous CT-guided fine-needle aspiration biopsies (CT-FNA). 13% of CT-FNAs were complicated by a moderate-to-large pneumothorax requiring ICC or hospitalization. Overall 16% of biopsies were for benign disease (117). Using a volumetric-based protocol, NELSON reported the surgical diagnostic procedure rate as 1.2% in round one and 0.8% in round two; 32/92 (35%) and 13/61 (21%) procedures in each round were for benign disease. Very few CT-FNAs were performed: 5/13 CT-FNA in round one and 3/3 FNA in round 2 showed benign disease. Across both rounds bronchoscopy diagnosed cancer in 111/247 (45%) procedures-a lower than expected figure likely reflecting peripheral tumour location. Complication rates were not reported (50).

The PLuSS study (118) screened 3,642 participants using an in-house protocol. 82 (2.3%) underwent surgical procedures (thoracotomy or VATS), twenty-eight of whom (34%) had benign disease. The study investigators cited "an apparent community bias toward aggressive intervention" for indeterminate lung nodules.

At baseline, 27.3% in the NLST LDCT group had a positive scan result (13). 155/7,191 participants had a percutaneous diagnostic procedure (CT-FNA in 120) and 297 (4.1% of positive scans) had a diagnostic surgical procedure (thoracotomy, thoracoscopy, mediastinoscopy or mediastinotomy) including 197 thoracotomies. Across all three screening rounds (75,126 screenings), 164/673 (24%) of surgical procedures in the LDCT group resulted in a non-cancer diagnosis. 191/673 (29%) of participants whose most invasive diagnostic procedure was surgical experienced at least one complication; in 80 (12%) this was classified as major. Only 14 of 99 (14%) participants who underwent a needle biopsy as their most invasive diagnostic procedure experienced one or more complication and none were major. 16 participants (10 with lung cancer) died within 60 days of an invasive diagnostic procedure, but it is not known whether death resulted directly from the diagnostic procedure. Put differently, 33 per 10,000 screenees suffered major complications during any diagnostic evaluation, but complications following bronchoscopy or needle biopsy were low, 1.5 and 0.7 per 10,000 screenees respectively; the frequency of death occurring within 2 months of a diagnostic evaluation was 8 per 10,000 (16). I-ELCAP

has not reported its rates of diagnostic procedures or complications.

CT-FNA appears safe with a complication rate of 13-14% and good concordance of biopsy result with resected pathological specimens histology (119). Bronchoscopy on the other hand, although safe, may have a lower yield for small, peripheral cancers detected by screening, although newer techniques such as endobronchial ultrasound and electromagnetic navigation may be able to improve yield (120,121). Surgical procedures have major complication rates of 12% but around 20-35% of cases are ultimately diagnosed with benign disease. This has an impact on cost-effectiveness.

Although ultimately the decision to resect an indeterminate nodule is a clinical one, given the high proportion of reported benign disease detected by screening, a positive tissue diagnosis prior to surgical resection is desirable. As demonstrated by the NELSON study, definite growth over a three month interval was due to benign disease in up to one third of cases. To date most studies have been run from expert tertiary centres where CT-FNA is available as the initial diagnostic procedure for small peripheral lesions. It is likely that strict governance and quality assurance will be needed to keep unnecessary biopsies and resections to a minimum.

Lung-preserving surgery

As reviewed by Blasburg *et al.*, evolving surgical technique, the recognition of good prognosis for small tumours, especially with a high GGO component, and the on-going risk of subsequent tumours, has turned attention to 'lung preserving' surgery (anatomical segmentectomy and wedge resection) as an alternative to lobar resection for small tumours (122). Two randomized controlled trials which will hopefully be able to answer this important question are currently recruiting [CALGB 140503 and JCOG0802/WJOG4607L (123)].

Quality of life (QoL)

Three studies have reported generic health-related QoL (HRQoL), anxiety and lung-cancer specific distress data from approximately 2,500 screening participants (124-126). All found some transient negative psychological effects for participants who received an indeterminate or suspicious screening result. These effects subsided fairly rapidly such that there were no significant differences in HRQoL between

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baseline and 12-24 months follow-up. The NELSON study reported that half the participants found waiting for their baseline CT scan results 'discomforting', but that an indeterminate result at the second round of screening had no impact on HRQoL. This suggests that minimizing the waiting time for test results is beneficial and that participants soon accept that an indeterminate scan result does not necessarily warrant high anxiety (124,127).

Smoking cessation

Smoking cessation is important not only for future risk reduction in participants without cancer, but may also improve the prognosis of those diagnosed with early stage lung cancer (128). Screening for lung cancer may be a "teachable moment" increasing motivation to quit, particularly if the participant receives an abnormal CT scan report (129-131). As successful smoking cessation programs may also make screening more cost-effective (99), and smoking cessation assistance 'adds value' to screening in several ways, it should be a core component of any lung cancer screening program.

Knowledge gaps

Despite the positive result from NLST, screening outside of a research trial should be conducted in a controlled environment with careful risk assessment prior to recommending screening and careful analysis of all outcomes to ensure quality. Two international workshops have considered the current state of evidence and future directions for research. Areas that need addressing were highlighted including: (I) how to optimise identification of high-risk individuals; (II) Screening protocols (e.g., screen interval, number of screening rounds); (III) Definition of a positive screen result; (IV) Management of indeterminate nodules; (V) Diagnostic and therapeutic interventions for suspicious nodules; (VI) Integrated smoking cessation programs; (VII) The role of early detection biomarkers in individual lung cancer risk assessment; (VIII) The rate of overdiagnosis. Important steps will be to standardise equipment and image quality, nodule analysis and interpretation, and participant follow-up and outcome reporting (93,132). Some of these areas are discussed below.

Overdiagnosis

Overdiagnosis is difficult to ascertain (see Box 2 for

definition). It was estimated at 13% in the NLST-the relative difference between 1,060 cancers detected in LDCT arm and 941 cancers detected in control arm (13). However this figure has been criticised as an underestimate (133) on the basis that the appropriate denominator should be the number of lung cancers detected in the control group during the screening period (n=470), not at the end of follow-up (n=941), making overdiagnosis closer to 25%, a figure similar to that estimated by the Mayo LDCT study on the basis of VDT (37). However even this figure may be an underestimate if the CXR screening arm is also subject to overdiagnosis (133). Against this, subset analysis of the PLCO cohort who met NLST eligibility criteria (n=30,321) found similar numbers of lung cancer cases in the CXR and the non-screened arms (518 *vs.* 520 cancers respectively after 6 years' follow-up) (12). It is likely that only the European trials comparing screening to usual care (i.e., no screening) will be able to give a true estimate of overdiagnosis (90). This question therefore remains unanswered at present.

Screening interval and length of follow-up

The appropriate screening interval should provide a favourable ratio between disease control and screening costs (134). The MILD trial recently published their findings from a three-arm RCT of observation *vs.* annual *vs.* biennial screening in 4,099 participants (89). Stage distribution and resection rates were similar in the two LDCT arms. The cumulative 5-year lung cancer incidence was highest in the annual LDCT group compared to biennial and control groups (620/100,000 *vs.* 457 and 311 respectively, P=0.036). Adherence to the screening protocol was >95% in each LDCT arm but median duration of follow-up was only 4.4 years. Recruitment fell significantly short of the planned 10,000 participants meaning the study was underpowered to detect mortality differences. Also, differences in characteristics of screened and non-screened groups (such as smoking status, smoking intensity and lung function) raise doubts about the adequacy of randomization (135). Long-term follow-up results from this study may be more informative. The NELSON study, in which participants are screened at Year 1 (baseline), Year 2 and Year 4, i.e., a two-year gap between the second and third scan, could also inform on optimal screen interval when Year 4 results are reported. As previously mentioned, data gathered at baseline scan (i.e., presence of radiographic emphysema) may be useful in determining risk and thus optimal screening

interval (68). Regarding duration of screening, the NLST LDCT arm detected 649 cancers after a positive screening test (270 at baseline and 168 and 211 at years 1 and 2 respectively) and 367 in participants who either missed the screening or were diagnosed after completing the trial screening phase (median follow-up 6.5 years). This suggests that cancer detection rates (i.e., cancer risk) do not drop significantly over time and that on-going screening may be required. Accordingly, current guidelines suggest annual screening until the age of 74 (14,16) or 79 (15).

Recruitment

Recruitment strategies have varied between studies, most commonly direct mailing and/or media releases, but some used general practitioner referral (84,88). Smokers, by definition are less risk averse than non-smokers, at least in terms of their health. The decision to enter a screening trial is a complex balance of factors including acceptability of screening methods, risk perception, altruism, and self-interest (136). Inevitably, volunteers in any trial are self-selected and contribute to the 'healthy volunteer' effect. This may result in overly optimistic outcomes (e.g., better screening compliance, higher smoking cessation rates) or overly pessimistic outcomes (e.g., lower effectiveness as lower-risk individuals benefit less from screening).

Both the NLST and NELSON studies found some differences between their study populations and eligible general population; Participants were younger and less likely to be current smokers and had higher education levels (a proxy for socio-economic status). These differences were considered minor, meaning that a significant healthy volunteer effect was unlikely (81,137).

Risk stratification

Risk stratification has been applied at a basic level with most studies adopting the ELCAP strategy of screening older persons with a smoking history. Although age and tobacco smoke exposure account for the vast majority of lung cancer risk it is well recognised that other risk factors such as family history, socioeconomic status, occupational exposure and COPD contribute (138). Further risk stratification using other readily available information may be able to improve screening efficiency by excluding lower risk participants (139). Various models have been proposed, the largest derived from PLCO Trial data and recently updated

(140,141). A retrospective analysis of this model applied to the PLCO dataset found that it was more efficient in comparison to the standard age- and smoking-based NLST entry criteria improving sensitivity from 71% to 83% (P<0.001), positive predictive value from 3.4% to 4.0% (P=0.01), and maintaining specificity (63% each). Use of the risk model to select screenees would have missed 41.3% fewer lung cancers (141). Prospective evaluation of another risk model is being undertaken by the UK Lung Cancer Screening Trial (142). Risk stratification may enhance screening effectiveness and cost-effectiveness by increasing lung cancer prevalence and incidence and reducing false-positive scan results. Although risk stratification makes intuitive sense it has not been proven experimentally, thus screening guideline recommendations diverge [recommend use of published risk model (15), informal risk assessment (14), no recommendation (16)].

Screening implementation

Generalization of findings from tightly controlled trial situations to large-scale mass screening programs require uniform standards and high quality control in order to be able to accurately track and assess nodules over time (132). Lung cancer screening is more than simple provision of a CT service; It is as a long-term commitment requiring extensive infrastructure to allow for invitation and recruitment; quality improvement; workforce/facility capacity for screening, diagnosis and treatment; health professional training; participant information and support. On-going evaluation and monitoring of the program is essential to ensure high standards of care are met and delivered in a consistent and acceptable way (134,143).

Future research

Minimally invasive, inexpensive tests to identify individuals at highest risk of lung cancer most likely to benefit from screening or to distinguish benign from malignant screen-detected nodules would represent major advances in lung cancer screening. Promising new technologies in this regard include analysis of blood for circulating microRNAs and exhaled breath for volatile organic compounds (144-146). Most recent LDCT screening studies included biomarker collection in their protocols, so we can expect exciting new insights into these areas in the near future.

Conclusions

The results of the landmark NLST have proven the long-held belief that screening for lung cancer can save lives. Understandably, as a new intervention, many questions remain making generalizability to non-US settings difficult. Over the next few years, further analysis of NLST data and maturation of other important trials will be able to fill these knowledge gaps allowing the lung cancer community to evolve and refine the way we screen.

Acknowledgements

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A review of current and novel therapies for idiopathic pulmonary fibrosis

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Abstract: Idiopathic pulmonary fibrosis (IPF) is a progressively fibrotic interstitial lung disease that is associated with a median survival of 2-3 years from initial diagnosis. To date, there is no treatment approved for IPF in the United States, and only one pharmacological agent has been approved outside of the United States. Nevertheless, research over the past 10 years has provided us with a wealth of information on its histopathology, diagnostic work-up, and a greater understanding of its pathophysiology. Specifically, IPF is no longer thought to be a predominantly pro-inflammatory disorder. Rather, the fibrosis in IPF is increasingly understood to be the result of a fibroproliferative and aberrant wound healing cascade. The development of therapeutic targets has shifted in accord with this paradigm change. This review highlights the current understanding of IPF, and the recent as well as novel therapeutics being explored in clinical trials for the treatment of this devastating disease.

Keywords: Idiopathic pulmonary fibrosis/drug therapy; idiopathic pulmonary fibrosis/pathology; molecular targeted therapy; clinical trials

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common and predominantly lethal form of the idiopathic interstitial pneumonias, with an associated median survival of only 2 to 3 years (1). The etiology of this chronic and progressive fibrotic lung disease is by definition unknown, although potential risk factors such as cigarette smoking and other environmental exposures have been described (1). While the diagnosis of IPF remains one of exclusion, its definition and the approach to its detection have evolved over the past decade (1,2). There has been a shift in the understanding of the pathophysiology of IPF from one of a chronic inflammatory state to one of abnormal wound healing. Aberrant fibroblastic proliferation and accumulation of extracellular matrix (ECM) proteins such as collagen have been the focus of more recent therapeutic experiments for IPF (3). This review highlights the current understanding of IPF, and the therapeutic clinical trials recently completed or underway for this devastating disease.

Epidemiology

Incidence and prevalence

The incidence and prevalence of IPF have been difficult to define as the diagnostic criteria for this disease have changed over the years (4). A United-States population-based study published in 1994, reported the incidence of IPF to be 10.7 cases per 100,000 per year for men and 7.4 cases per 100,000 per year for women (5). In a study published in 2006 based on a United States healthcare claims database, the prevalence of IPF was between 14-42.7 per 100,000, depending on whether narrow or broad case-finding criteria was used (6). Most recently, in May 2012, a systematic survey of literature estimated the prevalence of IPF in the European Union to be 26 per 100,000. The findings of various studies on the incidence of IPF are summarized in *Table 1*.

Table 1 Incidence of IPF		
Country or region of study (data source)	Incidence per 100,000	Authors
United States (healthcare claims database)	6.8-16.3	Raghu et al. 2006 (6)
United Kingdom (primary care database including diagnostic and prescribing data)	7.44	Navaratnam et al. 2011 (7)
Greece (survey of pneumonology departments)	3.38	Karakastani et al. 2009 (8)
Finland (hospital diagnostic coding databases)	16-18	Hodgson et al. 2002 (9)
European Union (review of medical literature)	26	Orphanet 2012 (10)
Permission has been obtained from John Wiley and Sons for reuse of figure *Table 1*.		

IPF represents the most common cause of death from progressive lung disease. Retrospective studies suggest that the median survival after diagnosis of IPF is 2-3 years, however, the course of IPF is variable, with some patients experiencing long periods of stability while others have frequent exacerbations or a rapid decline (1,11,12).

Age

IPF is more commonly seen in patients between 40 to 70 years of age (13). The incidence of this disease increases with age, and approximately two-thirds of those with IPF are older than 60, with a mean age at diagnosis of 66 years (7,13). The risk of death as a result of IPF also increases with age (1,7), with a hazard ratio (HR) of 0.25 for patients younger than 50 years (14) and a longer median survival amongst those younger than 50 (116.4 months compared to 62.8 months) (15). However, it has been suggested that this finding (i.e., younger age conferring longer survival from the time of IPF diagnosis) may be due to the inclusion of subjects with other types of interstitial pneumonias or varying definitions of disease onset in the older studies reporting these results (16). Nevertheless, age-related changes affecting cell regulation are likely important in the development of IPF (17).

Sex and race

IPF occurs more commonly in men than in women and may also progress faster and result in worse survival in men (18-20). Differences in disease progression, however, do not completely explain better survival in women (18). The IPF mortality rate in the United States was found to be 61.2 deaths per 1,000,000 in men and 54.5 per 1,000,000

in women (21), nevertheless, the death rate in women is increasing at a faster rate than in men (21). Age-adjusted mortality has been found to be greater among whites than blacks and is increasing at a higher rate among whites when compared to other racial and ethnic groups (21). Age-adjusted mortality among Hispanics has also been found to be lower than white non-Hispanics (21). Race and ethnicity may thus play a role in the susceptibility to IPF.

Diagnosis

The diagnosis of IPF requires the consideration of a detailed clinical history of the patient, a thorough investigation and exclusion of known causes of interstitial lung disease (e.g., domestic and occupational environmental exposures, connective tissue disease, and drug toxicity), radiographic concordance and, in certain cases, pathological confirmation by surgical lung biopsy (1).

The histopathological criteria for IPF are those of usual interstitial pneumonia (UIP). Within the lungs, UIP is a temporally and geographically heterogeneous mixture of fibrosis, scarring and honeycombing along with areas of less affected or unaffected parenchyma. The subpleural and paraseptal parenchyma are more severely affected (1,2). It is important to note that UIP is not unique to IPF, and that other interstitial lung diseases such as chronic hypersensitivity pneumonitis, some connective tissue diseases, and pneumoconioses such as asbestosis may reveal this histopathology as well (1,2).

A specific UIP pattern has been described with respect to high-resolution computerized tomography (HRCT) of the chest (1). This entails the presence of reticular opacities with a subpleural basal predominance, honeycombing with or without traction bronchiectasis, and the absence of features that coincide more with other known forms

of interstitial lung disease such as ground glass opacities, mosaic attenuations, and cystic disease (1). Overall, the positive predictive value of an HRCT diagnosis of UIP ranges from 90-100% (1). A consensus has evolved that surgical biopsy is usually not required when patients have clinical and radiographic features that fit the accepted UIP pattern (22).

In summary, the diagnosis of IPF requires: (I) exclusion of other known causes of interstitial lung disease, (II) the presence of an UIP pattern on HRCT in patients not subjected to surgical lung biopsy, and (III) specific combinations of HRCT and surgical lung biopsy pattern in patients subjected to surgical lung biopsy (1).

Pathogenesis

Great advances have been made in the understanding of the pathogenesis of this disease and with this, a hope of a more targeted approach in therapy has emerged. Borchers *et al.* describe the research efforts that have focused on better understanding the reasons for an increased presence of fibroblasts in IPF lungs (23). The prevailing hypothesis is that UIP histology stems from repeated epithelial injury leading to the activation of alveolar epithelial cells (AECs) (23). These AECs then attract and activate fibroblasts and induce fibroblast proliferation and differentiation into myofibroblasts. Improper re-epithelialization leads to continued accumulation of myofibroblasts and their production of an excess of extracellular matrix. A possible role of humoral immunity, and autoimmune reaction, as well as genetic influences gleaned from those with familial IPF have also been described (23).

In light of these advances, IPF is not currently viewed as a purely inflammatory disorder. Rather, UIP is seen as a state of abnormal wound healing (11,24), "with progressive extracellular matrix accumulation, decreased fibroblast-myofibroblast cell death, continuous epithelial cell apoptosis, and abnormal re-epithelialization."(11) It is likely for this reason that broad anti-inflammatory and immunosuppressive therapies have not been able to alter this progressively fatal disease. Selman and colleagues propose that future treatments for IPF must be directed at crippling the fibroproliferative response and promoting normal alveolar re-epitheliazation (11).

The role of an inflammatory response in the pathogenesis IPF remains, however, and has been highlighted by recent work with murine lung injury models. These data support the pathogenic role of an early inflammatory response involving danger signals in the form of uric acid production; with an attenuation in observed fibrosis following the administration of agents to reduce tissue uric acid levels (25). Elevated uric acid levels have also been observed in human IPF lungs as compared to non-fibrotic lungs (26). While the expression of genes associated with acute inflammatory pathways has not been found to be increased in IPF, several genes encoding for chemokines and cytokines are upregulated (27). Therefore, consideration of more finely tuned anti-inflammatory therapies such as the selective modulation of key inflammatory pathways has also been proposed (28).

A history of treatment strategies

Unsuccessful treatments to date

Anti-inflammatory/immunomodulatory agents
Corticosteroid monotherapy
Corticosteroids such as prednisone suppress cellular and humoral immunity, reducing the levels of pro-inflammatory molecules. As IPF was initially considered a primarily inflammatory disease, broad immunosuppression was considered as a potential therapy. A 2003 Cochrane database analysis that was assessed as up-to-date in 2008, concluded that there have been no adequate randomized controlled trials to assess the efficacy of corticosteroid monotherapy in IPF (29). Furthermore, the use of chronic corticosteroids has been shown to be associated with a significant number of co-morbidities (30) and controlled cohort studies have revealed no survival benefit among those treated with corticosteroids (31). Given the advances in our understanding of the pathophysiology of this disease, trials with corticosteroid monotherapy are no longer justified and their sole use in IPF is not recommended in the more recently published consensus statement (1).

Azathioprine
Azathioprine, an immunosuppressant that blocks the function of proliferating cells such as T cells and B cells and also decreases the number of circulating monocytes and granulocytes, has long been considered as potential therapy for IPF. The use of azathioprine plus prednisone was associated with an improvement in lung volumes and gas exchange in a small retrospective study in 1978 (32). When analyzed prospectively in a randomized double-

blind controlled study (azathioprine/prednisone versus prednisone/placebo), there was a trend towards survival benefit in the treatment arm, though it did not meet statistical significance (33). Interpretation of these studies is made difficult as they include the use of older, less defined diagnostic criteria for IPF that have since changed (34). Azathioprine in combination with prednisone was more recently prospectively evaluated for IPF in a randomized, placebo controlled, double-blind trial (clinicaltrials.gov identifier NCT00518310). The results of this latter study have not yet been published and the use of azathioprine along with corticosteroids is not currently recommended.

Cyclophosphamide

Cyclophosphamide, a cytotoxic chemotherapeutic agent, has been evaluated as a therapy for IPF in combination with prednisone. While no prospective, randomized trials of this drug combination exists, two retrospective reports are available. In one study of 82 patients, a survival advantage was observed among those treated with prednisone/cyclophosphamide versus those with prednisone monotherapy, however, this applied only to those with less severe disease as measured by forced vital capacity (FVC ≥70%) (35). Collard *et al.* reviewed the use of corticosteroids plus cyclophosphamide compared to no pharmacotherapy in a larger (n=164) retrospective controlled study and found no significant difference in mortality between the two (36). Therefore, current recommendations advise against the treatment of IPF with a combination of corticosteroids and immunomodulator therapy (1).

Everolimus

Everolimus, a derivative of rapamycin, is a macrocyclic proliferation signal inhibitor with immunosuppressive and anti-fibroproliferative properties, currently used as immunosuppressant to prevent transplant rejection (37). By arresting the cell cycle at the G1 to S phase, everolimus inhibits growth factor-dependent proliferation of hematopoietic and non-hematopoietic cells such as vascular smooth muscle cells and human adult lung fibroblasts (37). Everolimus has been observed to attenuate bleomycin-induced pulmonary fibrosis in the rat model. Its safety and efficacy in the management of IPF was recently assessed in a randomized, placebo-controlled 3-year study of 89 patients (Australian New Zealand Clinical Trials Registry number ANZCTR 12605000599673). Everolimus was associated with a more rapid disease progression (mean time to disease progression defined as deterioration in pulmonary function =180 days) when compared to the placebo group (mean =450 days to disease progression) (37). The authors note that a higher dose of everolimus (8 mg) was used than that usually administered in solid organ transplantation. Nearly half (48%) of patients in the treatment arm were unable to tolerate this initial dose due to side-effects, and 23% of patients in the everolimus group discontinued the drug for this reason (37). While the fact that 68% of subjects randomized to everolimus overall had stopped the study drug by 12 months compared with only 12% of subjects randomized to placebo (38) makes interpretation of results difficult, it is concluded that everolimus, despite its immunosuppressive and anti-fibroproliferative properties, has not proven effective in the management of IPF, and may in fact be harmful.

Anticoagulants and the coagulation cascade

Repetitive and widespread injury to the alveolar epithelium is considered to be the pathogenic force behind IPF. Wound repair involves the activation of the coagulation cascade, inflammatory cell recruitment and the formation of a provisional matrix to prevent blood loss (39). In the fibrotic lung, tissue factor (40) and thrombin (41) are highly expressed, while the activation of protein C is decreased, resulting in an increase in procoagulant activity in the alveolar spaces (42) as well as abnormal collagen turnover within the alveoli (43).

Prophylactic or therapeutically administered anticoagulants are effective in ameliorating fibrosis in animal bleomycin models (44,45). The use of anticoagulants has therefore been evaluated among patients with IPF.

Warfarin/heparin/prednisolone

Kubo and colleagues published a non-blinded randomized trial of 56 patients with IPF in Japan (46). Patients were assigned to receive prednisolone only or prednisolone plus anticoagulant therapy (oral warfarin or low-molecular weight heparin). They reported significantly increased mortality in the non-anticoagulant group compared to the anticoagulant group (HR=2.9) after 3 years of therapy. Mean plasma levels of D-dimer were significantly higher in patients who died from AE (3.3 *vs.* 0.9 mcg/mL) (46). However, limitations of this study include its unblinded design, as well as a 26% withdrawal rate in the anticoagulant group (47). To further investigate the utility of anticoagulation for patients with IPF, the National Heart, Lung and Blood Institute (NHLBI) conducted the AntiCoagulant Effectiveness in Idiopathic

Pulmonary Fibrosis (ACE-IPF) trial, a double-blind randomized study comparing the administration of warfarin versus placebo in this patient population (clinicaltrials.gov identifier NCT00957242). This trial was terminated due to excess mortality in the warfarin arm (14 warfarin *vs.* 3 placebo deaths, adjusted HR=4.85), and a low probability of treatment benefit. While no significant treatment effects in quality of life measures or physiologic endpoints (FVC, 6-minute walk distance, or DlCO) were observed, higher rates of hospitalization and AE IPF were noted in the warfarin arm (48). A review by an independent Data Safety Monitoring board concluded that warfarin is unlikely to prove superior to placebo as a therapy in IPF (48). Recently, the tolerability of inhaled heparin in IPF was investigated in a small open-label pilot study under the premise that direct administration of this drug would not be associated with untoward systemic side effects of anticoagulation. No adverse effects of alveolar anticoagulation with nebulized heparin were noted in this trial (49).

Endothelin receptor antagonists and vasodilators

Animal and subsequent human studies have suggested that endothelin-1 plays a significant role in IPF (50), as it has been found to promote fibroblast proliferation (51,52), myofibroblast differentiation (52), collagen synthesis (53), and endothelial cell mitosis (54). Further, bleomycin-induced lung fibrosis in rats leads to an increase in endothelin-1 as well as increased expression of its receptor (50), and in humans, endothelin-1 has been found to be expressed at higher levels in the lung tissue of IPF patients when compared to their control counterparts (55,56).

Bosentan

The endothelin receptor antagonist, bosentan, has recently been the subject of considerable investigation. Bosentan was the subject of two large phase III blinded, randomized trials, known as the BUILD-1 and BUILD-3 studies, into which a total of 774 subjects were enrolled (clinicaltrials.gov identifier NCT00071461 and NCT00631475, respectively). Unfortunately, neither study was able to meet its primary endpoint [change in 6 minute walk test distance by month 12 for BUILD-1 (57), and death or disease progression defined by a decline >10% in FVC and 15% in DlCO or an acute exacerbation of IPF at month 12 for BUILD-3 (58)]. While this was a well-tolerated therapy, its failure to result in significantly improved outcomes makes this a non-viable treatment option for IPF at this time (57,58).

Ambrisentan and macitentan

Other endothelin receptor antagonists, macitentan and ambrisentan, have recently been evaluated in phase II double-blind, randomized placebo controlled studies (clinicaltrials.gov identifier NCT00903331 and NCT00768300, respectively). The macitentan trial, known as the MUSIC study, enrolled 178 patients with IPF but did not meet its primary endpoint of forced vital capacity and therefore a phase III study will not be initiated. The ambrisentan trial, known as ARTEMIS-IPF, was terminated by the sponsor after an interim analysis of unblinded efficacy and safety data did not show evidence of a treatment benefit (59). Further details on these two studies have yet to be published.

Sildenafil

Sildenafil, a phosphodiesterase type-5 (PDE5) inhibitor, is today approved for use by the United States Food and Drug Administration (FDA) for idiopathic pulmonary artery hypertension (PAH) (60). Sildenafil stabilizes the second messenger of nitric oxide, cyclic guanosine monophosphate, leading to pulmonary vasodilation (61). Given that this drug seems to preferentially induce vasodilation in well-ventilated lung tissue, it is presumed that it can improve ventilation-perfusion matching (and therefore gas exchange) in IPF (61). Over one-third (33-50%) of patients with IPF undergoing formal lung transplantation evaluation have been noted to have PAH at rest as diagnosed by right heart catheterization, and the presence of PAH in those with IPF portends a poorer survival (62,63). In a small study of 14 patients with IPF (clinicaltrials.gov identifier NCT00352482), the oral administration of 25-50 mg of sildenafil three times daily for three months led to a mean improvement in 6MWD of 49.0 meters (90% confidence interval 17.5-84 meters) (64). A pilot study was thus created to further explore the potential benefit of sildenafil in IPF (clinicaltrials.gov identifier NCT00359736). Twenty-nine patients with moderately impaired pulmonary function and estimated ventricular systolic pressures (or pulmonary artery systolic pressures) of 25-50 mmHg, were randomly assigned to this double-blind, placebo-controlled study. There was unfortunately no significant improvement in 6MWD distance or dyspnea score in the sildenafil treatment group (65). Finally, 180 patients with IPF were randomized to receive oral sildenafil or placebo in a large double-blind, placebo-controlled trial called STEP-IPF (clinicaltrials.gov identifier NCT00517933) (61). The primary outcome measure (20% of the 6MWD at 12-weeks) did not meet statistical

significance as only 10% in the sildenafil arm versus 7% in placebo arm showed improvement (P=0.39). There were however small but clinically significant differences in the secondary outcomes of arterial oxygenation, DLCO, degree of dyspnea and quality of life in those receiving sildenafil. Of note, data regarding the right-heart catheterization were not available in this study, thus, the presence and degree of pulmonary arterial hypertension is unknown among this study population (66). To date, there is not enough evidence to routinely support the use of sildenafil in IPF.

Antifibrotics and cytokine/kinase inhibitors
Interferon-gamma

IPF appears to be characterized by a predominantly T-helper cell type 2 cytokine state. In fact, it is very likely that the progression from inflammation to fibrosis is caused by a shift from a T-helper cell type 1 to type 2 cytokine profile state, thereby activating fibroblasts and ECM deposition and remodeling (24). Interferon-gamma (IFN-gamma) is an immunoregulatory cytokine that directly limits fibroblast proliferation and collagen synthesis. Use of IFN-gamma may actually revert the balance to one of a predominantly T-helper type 1 cytokine state (67).

IFN-gamma administration in mice has been shown to diminish bleomycin-induced lung fibrosis (28,68). In humans, a preliminary trial randomized 18 IPF patients to receive either a combination of IFN-gamma and prednisone versus prednisone only. Total lung capacity improved among those receiving IFN-gamma (from 70±6% of the predicted value at base line to 79±12% at 12 months, P<0.001 for the difference between the two groups) (69). However, a more recent study of IFN-gamma, the INSPIRE trial, did not find a significant benefit to IPF patients with respect to their primary outcome of survival (clinicaltrials.gov identifier NCT00075998). This randomized double-blinded placebo-controlled trial enrolled 826 patients with IPF to receive either IFN-gamma or placebo three times weekly (70). At their second interim analysis, the hazard ratio for mortality in patients on IFN-gamma showed no benefit compared with placebo, and the study was closed. After a median duration of 64 weeks on therapy, 15% of patients on IFN-gamma and 13% of patients on placebo had died (70). IFN-gamma is currently not recommended for the treatment of IPF.

Interest in IFN-gamma has not been lost, however, and it has been hypothesized that this compound may prove effective if delivered directly to the epithelial lining of the lung. A small pilot study involving inhaled aerosolized IFN-gamma over an 80-week period in 10 IPF patients was recently completed (clinicaltrials.gov identifier NCT00563212). Aerosolized IFN-gamma was well-tolerated and associated with a minimal change in FVC; a trend toward decreased decline in TLC and DLCO was observed post therapy (71).

Etanercept

Tumor necrosis factor (TNF)-alpha is highly expressed in the lungs of individuals with IPF, and functional polymorphisms of this cytokine are linked to an increased risk of developing IPF (28). TNF-alpha has both inflammatory and fibrogenic properties. In mouse models, for example, the injection of anti-TNF-alpha antibodies diminishes bleomycin-induced pulmonary inflammation and fibrosis (72). Furthermore, the overexpression of TNF-alpha has been found to increase fibroblasts and deposition of ECM proteins in the pulmonary interstitium (28,73). A randomized, placebo-controlled phase II trial of 65 patients with IPF was therefore conducted to assess the safety and efficacy of Etanercept, a recombinant human TNF-alpha receptor that binds to and inactivates TNF-alpha (clinicaltrials.gov identifier NCT00063869) (74). After 48 weeks of treatment, however, there was no significant improvement in the primary endpoints (change in FVC, DLCO, and $P(A-a)O_2$ at rest). Thus, the use of etanercept in patients with IPF is not advised (74).

Imatinib

The potential use of the platelet-derived growth factor receptor (PDGFR), a mitogen and chemo-attractant for mesenchymal cells such as myofibroblasts (47,75), has been studied in IPF. PDGFR mRNA is also increased in the lungs of those with IPF (76). Imatinib, an anti-proliferation protein tyrosine kinase inhibitor of PDGFR and c-kit, has prevented fibrinogenesis in bleomycin-induced fibrosis, and attenuated radiation-induced and asbestos-induced fibrosis in murine models (76). In IPF, however, a clinical phase 2 study of imatinib versus placebo (n=119) found neither a survival benefit nor effect on FVC (clinicaltrials. gov identifier NCT00131274) (77). Imatinib was also evaluated in a phase I/IIa trial in patients with systemic sclerosis-associated ILD; the drug was discontinued in 5 out of 20 patients due to adverse effects including generalized rash, diarrhea, transaminitis, myopathy, and possibly new diastolic heart failure (78).

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Activation of the stress-activated protein kinase, c-Jun N-terminal kinase (JNK), in epithelial and endothelial cells is associated with worsening fibrosis and increased inflammatory cytokine expression in IPF lungs (79). JNK induces tissue factor expression (80), which in turn drives thrombin production and fibrin generation (81). Inhibition of JNK in human lungs fibroblasts prevents differentiation to the myofibroblast phenotype by Transforming Growth Factor-beta1 (TGF-β1) (82). Bleomycin induces epithelial cell death through a JNK-dependent mitochondrial death pathway in rodents (83), and mice lacking JNK are protected against TGF-β1 and bleomycin-induced lung fibrosis (84). Inhibition of JNK could therefore potentially serve a therapeutic end in IPF. The safety of CC-930, an antifibrotic inhibitor of JNK (85), was recently tested in a phase II clinical trial (clinicaltrials.gov identifier NCT01203943). The trial, however, was terminated by the sponsor, citing that the benefit/risk profile did not support its continuation as rationale for its early end (86).

Treatments under investigation

Antioxidant/immunosuppressant/antiiflammatory therapies
N-acetylcysteine

N-acetylcysteine (NAC) is a precursor to the antioxidant glutathione. Glutathione has been found to be depleted in the lungs of those with IPF (4). NAC has been viewed as a potentially effective therapeutic regimen in IPF in the hope that repletion of glutathione stores would restore natural oxidant/anti-oxidant balance to prevent the oxidative injury that precedes fibroproliferation (4). In a non-randomized prospective study of 18 patients, the addition of NAC to the current therapy (corticosteroid ± immunomodulator) revealed improved lung function measures (87). The use of NAC was thus explored prospectively with 155 patients randomized to receive treatment (NAC) or placebo in addition to prednisone and azathioprine in the IFIGENIA trial (clinicaltrials.gov identifier NCT00639496) (88). At 12 months, the use of NAC slowed down the decline in vital capacity (relative difference of 9%, P=0.02) and DLCO (relative difference of 24%, P=0003). There was however no survival benefit in the treatment arm. Of note, this study had a large drop-out rate and, by 12 months, 30% of patients had died or were lost to follow-up.

The NHLBI designed the ongoing PANTHER-IPF trial to evaluate the effectiveness of the combination of prednisone, azathioprine, and N-acetylcysteine (NAC) *vs.* NAC alone *vs.* placebo (clinicaltrials.gov identifier NCT00650091) (66). After 155 of the 390 planned patients were enrolled, a data safety monitoring committee recommended that the combination treatment arm of this trial be stopped. This was based upon the discovery that, when compared to placebo, the three-drug regimen led to a significant increase in mortality (11% *vs.* 1%), hospitalizations (29% *vs.* 8%) and serious adverse events (31% *vs.* 9%), and did not show an improvement in pulmonary function (89). While recent IPF treatment guidelines listed this three-drug combination therapy as a weak recommendation (1), it had until very recently been commonly viewed as the default standard of care for IPF. Subjects in the PANTHER-IPF trial arms receiving NAC alone and placebo continue to be followed. To date, there exist too few data to recommend NAC monotherapy in IPF (1).

Antifibrotic/antiinflammatory/antioxidants
Pirfenidone

Pirfenidone, an orally administered pyridine, is the only drug approved for clinical use in the treatment of IPF worldwide (90). It is an anti-inflammatory and antioxidant agent that inhibits transforming growth factor-β in vitro (91). Pirfenidone also acts as an antifibrotic by directly altering the expression, synthesis, and possibly accumulation of collagen, and inhibiting the recruitment, proliferation and possibly expression of the extracellular matrix-producing cells (90). Based on favorable results in two open-labeled compassionate use studies followed by a Japanese phase II trial, three randomized, double-blind, placebo-controlled, multicenter, phase III studies were conducted. Two of these were the almost identical multinational 004 and 006 trials (referred to as the CAPACITY studies), and the third trial was conducted in Japan (92,93).

In the 004 trial, 435 patients with IPF were assigned in a 2:1:2 dosing ratio to 2,403 mg/day pirfenidone, 1,197 mg/day prifenidone, and placebo (92). In the 006 study, 344 patients were assigned to either 2,403 mg/day of pirfenidone or to placebo. In study 004, pirfenidone reduced the decline in FVC (P=0.001), with a mean reduction at 72 weeks of 8% (SD 16.5) in the 2,403 mg/day group and a reduction of 12.4% (SD 18.5) at 72 weeks in the placebo group (difference 4.4%, 95% CI, 0.7 to 9.1). However, in study 006, the change in FVC at 72 weeks was not significant between the treatment and

placebo arms (P=0.501) (92). It was largely based on these studies that pirfenidone was approved by the European Commission (EC) in 2011 (94) for the treatment of mild to moderate idiopathic pulmonary fibrosis.

In the double-blind, placebo-controlled randomized Japanese trial by Taniguchi *et al.*, pirfenidone was administered in a 2:1:2 ratio (1,800 mg/day, 1,200 mg/day or placebo) to a total of 275 patients over a 52 week period (93). The primary endpoint, a change in lung vital capacity, was significantly preserved in the higher dose versus placebo group (–0.09 *vs.* –0.16 L respectively, P=0.0416). Limitations to this study include the enrollment of a relatively homogeneous Japanese population, as well as the fact that the primary end-point was changed before unblinding. Furthermore, the change in primary endpoint was recommended by the members of the data safety and monitoring board after review of interim comparative data of the primary and secondary end points, possibly compromising the integrity and credibility of the trial (95). The study also observed a significant progression-free survival time (a secondary end-point) between these two groups (P=0.0280). In an exploratory analysis of this study later published by Azuma *et al.*, it was observed that a subpopulation of these patients (those with a baseline predicted VC ≥70% and oxygen saturation <90%) had a greater benefit from pirfenidone (96). A well-known side effect of pirfenidone, photosensitivity, was frequently observed in this study (51% of patients in the high-dose group and 53% in the low-dose group). Pirfenidone was approved in 2008 for use in the management of IPF in Japan by the Japanese Ministry of Health, Labour and Welfare, in part because of this study (97). Despite its approval by both the EU and Japan, due to concerns including a perceived lack of efficacy as measured by change in FVC, and lack of survival benefit, the use of pirfenidone for IPF has not been approved by the FDA (98). A new phase III trial of pirfenidone aiming to detect a clinically meaningful effect on forced vital capacity is therefore underway in the United States (the ASCEND trial, clinicaltrials.gov identifier NCT01366209).

Targeting cytokine networks involved in immune and structural cell activation
Inhibition of transforming growth factor-β (TGF-β)
In animal models, TGF-β, a pleitropic cytokine, is increased prior to collagen synthesis, and in lungs of individuals with pulmonary fibrosis, immunohistochemical staining reveals increased TGF-β, most notably in areas of regeneration and remodeling (99). TGF β exists in 3 isoforms in mammals (100), and a growing body of evidence suggests that one of these, TGF-β1, is a key pro-fibrotic agent. Its activity is characterized by the promotion of extracellular matrix production (101), fibroblast to myofibroblast differentiation (100), and inhibition of autophagy in fibroblasts (102). These insights have made TGF-β1 an important ongoing therapeutic target in IPF. A Phase I trial of GC1008, an antibody targeting all TGF-β isoforms, has recently been completed (clinicaltrials.gov identifier NCT00125385), however, the results of this study are not yet available.

TGF-β plays a key role in cellular homeostasis, acting as a tumor suppressor under certain circumstances (103). Because patients with IPF are at increased risk for developing lung cancer (104), the direct inhibition of TGF-β could potentially result in very undesirable side effects (28). The TGF-β activation cascade therefore poses a more attractive therapeutic target. Partial inhibition of αvβ6 integrin, a key activator or TGF-β1, has been shown to prevent bleomycin-induced pulmonary fibrosis without exacerbating inflammation in mice (105). A humanized monoclonal antibody against αvβ6 integrin, STX-100 is currently under evaluation in a randomized, placebo-controlled phase II IPF trial (clinicaltrials.gov identifier NCT01371305).

Other members of the TGF-β superfamily, bone morphogenic proteins (BMPs), are also involved in injury repair and homeostasis. Interestingly, a BMP antagonist, gremlin, is upregulated in IPF lung biopsies. In mice exposed to asbestos, those treated with BMP had reduced fibrosis. In fact, markers of collagen deposition in the lung were decreased by 50%, suggesting that the preservation of BMP activity may be of therapeutic value in IPF (106).
Inhibition of connective tissue growth factor (CTGF)
Connective tissue growth factor (CTGF), a matricellular protein, is thought to be a central mediator of tissue remodeling and fibrosis. It is highly expressed in IPF fibroblasts (107) and in bleomycin-challenged mice (108). CTGF is induced by TGF-β, and mediates some of the profibrotic effects of TGF-β (100); it also activates type-1 collagen expression (108). Anti-CTGF antibodies have been shown to decrease collagen-1 gene activity. In murine models of multiorgan fibrosis caused by the administration of CTGF and TGF-β and in bleomycin-induced lung fibrosis, the administration of a human CTGF antibody, FG-3019, results in reduced histological signs of

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fibrosis (109). Preliminary safety and efficacy data from an open-label, phase II trial of FG-3019 (clinicaltrials. gov identifier NCT01262001), were recently presented at the European Respiratory Society 2012 conference (110). While this trial is still ongoing and not all data have been analyzed, improvement or stability of fibrosis as determined by HRCT scan quantification was apparent in 14 of 25 IPF patients after 24 weeks of treatment with FG-3019, and this improvement was positively associated with changes in FVC (110). While these preliminary findings are promising and a randomized, placebo-controlled trial of FG-3019 is planned, it is important to note that the results of this study have not yet been published in a peer-reviewed publication.

Somatostatin analogues

Expression of receptors for somatostatin, a regulator of growth hormone secretion also known as a growth hormone-inhibiting hormone, is increased in human IPF lungs (111). The somatostatin analog SOM230 has been observed to have an antifibrotic effect in bleomycin-induced lung fibrosis in mice, resulting in a decreased expression of TGF-β and CTGF (112). Treatment with octreotide, another somatostatin analogue, has shown to decrease parenchymal fibrosis and structural deformities in the bleomycin model (113). Somatostatin analogues therefore merit evaluation as therapeutic agents for IPF. Octreotide was recently tested in a small non-randomized open-label study. Twenty-five IPF patients were enrolled to receive octreotide, and 17 completed the study, receiving treatment over a 48-week period (clinicaltrials.gov identifier NCT00463983). Compared to historical controls (subjects from other published IPF trials), the rate of decline in pulmonary function (FVC and DlCO) was lower in subjects treated with octreotide (114). Octreotide thus remains a potentially useful agent for the treatment of IPF; however, larger randomized, controlled trials are necessary to confirm this.

Inhibitors of IL-13, IL-4 and CCL2

Another driver of lung fibrosis is the cytokine expressed by T helper type 2 lymphocytes, interleukin-13 (IL-13), which, through the chemokine CCL2, upregulates TGF-β1 to stimulate fibrosis, as observed in a murine model. In humans, IPF fibroblasts are hyper-responsive to TGF-β1, IL-13, and to CCL2, and it has been suggested that these molecules may each mediate the function of the other in a pro-fibrotic manner. Inhibition of CCL2 orthologs resulted in reduced collagen deposition in an in-vivo bleomycin model. CCL2 is a known fibrocyte chemoattractant (107), further, high CCL2

levels may be correlated with progression of IPF (115). IL-13 has also been observed to stimulate collagen deposition and myofibroblast differentiation both independently and with the help of TGF-β1 (116). CCL2 and IL-13 therefore pose attractive therapeutic targets in IPF. Phase II trials of CNTO888 and QAX576, CCL2 and IL-13 antibodies, respectively, have recently been completed. The results of both trials are awaited (clinicaltrials.gov identifier NCT00786201 and NCT00532233). Tralokinumab, a human recombinant monoclonal antibody for IL-13 is currently being tested for IPF in a phase II randomized, placebo-controlled trial (clinicaltrials.gov identifier NCT01629667).

Interleukin 4, a cytokine structurally related to IL-13, has also been implicated in the abnormal proliferation of fibroblasts that characterizes IPF (117). Both IL-13 and IL-4 are elevated in the bronchial alveolar lavage fluid of IPF patients (118) and increased expression of the receptors that bind IL-4 and IL-13 has been detected in fibroblasts grown from surgical lung biopsies of patients with UIP as compared to those from patients with other idiopathic interstitial pneumonias and patients without lung fibrosis. Further, the proliferation of UIP-derived fibroblasts is inhibited when exposed to the cytotoxic effects of a Pseudomonas exotoxin targeting the IL-13 and IL-4 receptors, suggesting that fibroproliferation in UIP can be modulated by agents targeting these cytokines (119). To this end, a randomized, double-blind, placebo-controlled study of an engineered bispecific antibody targeting both IL-4 and IL-13, SAR156597 (120), is now enrolling patients with IPF (clinicaltrials.gov identifier NCT01529853).

Thalidomide

Although thalidomide is to blame for some of the most infamously tragic adverse effects in modern medicine, it has recently been used effectively for treating multiple myeloma and other conditions. Thalidomide is an anti-angiogenic (121), immunomodulatory (122), anti-inflammatory (123) drug. Thalidomide administration can attenuate fibrosis in bleomycin-challenged mice, possibly through the inhibition of TGF-β1-induced signaling pathways (124) and a reduction of vascular endothelium growth factor (VEGF) expression (125). An open-label study to determine the safety, feasibility and efficacy of this potential anti-fibrotic agent concluded in 2007, however, the results have not been published (clinicaltrials.gov identifier NCT00162760). Thalidomide also represents a promising therapeutic agent for a debilitating symptom

affecting nearly 80% of IPF patients that is refractory to current treatments: severe, persistent cough. Recently, a phase III randomized, double-blind study of 98 IPF patients with chronic cough demonstrated that thalidomide can improve cough and symptom-specific quality of life (126) (clinicaltrials.gov identifier NCT00600028).

Inhibition of LOXL2

The enzyme lysyl oxidase-like 2 (LOXL2) generates the scaffold on which fibroblasts grow by cross-linking fibrillar collagen. This enzyme is apparently over-expressed in IPF lungs and associated with activated fibroblasts, reactive pneumocytes, and vasculature in fibrotic foci (127). Inhibition of LOXL2 results in reduced levels of activated fibroblasts and TGF-β pathway signaling in human fibroblasts and bleomycin-treated mice (127). An allosteric inhibitor of LOXL2, the humanized monoclonal antibody GS-6624 (formerly AB0024), was evaluated in a phase I trial for the treatment of IPF (clinicaltrials.gov identifier NCT01362231), and a phase II trial is planned.

Targeting angiogenesis and ECM collagen deposition

While angiogenesis may exist as a mechanism to promote alveolar repair in fibrosing lung disease, its role may well be pathogenic in IPF. New blood vessel formation is regulated by angiogenic and angiostatic factors that respectively promote or inhibit neovascularization (128). Angiogenic chemokine expression is reportedly increased in IPF (129), and low levels of angiostatic chemokines have been observed in bleomycin-induced fibrosis (130). Paradoxically, the angiostatic chemokine, pigment epithelium-derived factor (PEDF), has also been noted to be elevated in IPF lungs. PEDF however is regionally associated with heterogeneous vascularization, characterized by a near absence of vessels within the fibroblastic foci, more prominent vascularity in the areas of fibrosis around the fibroblastic foci, and abnormal vessels in the most architecturally distorted regions (131). This heterogeneity may support fibroproliferation whilst inhibiting normal repair mechanisms (132).

Tyrosine kinase inhibitor BIBF 1120

BIBF 1120 is a tyrosine kinase inhibitor that suppresses pro-angiogenic intracellular signaling by targeting the proliferative growth factor receptors in fibroblasts (FGFR), platelets (PDGFR), and the vascular endothelium (VEGFR) (133). Blockade of these receptors may be therapeutic in IPF as their activation has been implicated in the pathogenesis of fibrosis (134-136). A phase IIb 12-month, randomized, double-blind, placebo-controlled study of BIBF 1120 was recently conducted to evaluate its safety and efficacy in IPF. This study, called the TOMORROW trial, demonstrated a trend toward a reduction in the decline in lung function, with fewer acute exacerbations and preserved quality of life in IPF patients (137). This prompted two currently ongoing, nearly identical phase-III randomized, placebo-controlled studies to further investigate the efficacy of BIBF 1120 in IPF (the INPULSISTM trials; clinicaltrials.gov identifiers NCT01335464 and NCT01335477).

Tetrahiomolybdate and minocycline

Administration of angiostatic chemokines and other agents with angiostatic properties such as tetrahiomolybdate, has been observed to reduce both angiogenesis and fibrosis in the bleomycin model (138-140). Minocycline hydrochloride, a broad-spectrum tetracycline antibiotic with anti-inflammatory and anti-angiogenic properties (141), was evaluated in a phase III clinical study of IPF patients (clinicaltrials.gov identifier NCT00203697). The safety of tetrahiomolybdate, was also evaluated in IPF in a phase I trial (clinicaltrials.gov identifier NCT00189176). Although both studies have concluded, their results are yet unknown (142).

Doxycycline

A key feature of IPF is the excessive deposition of extracellular matrix and basement membrane disruption that may be at least in part due to an imbalance between secreted matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) that results in a relative overexpression of TIMPs (143). In spite of their ability to break down the ECM, several MMPs (1 through 3 and 7 through 9) are paradoxically highly upregulated in IPF lungs (27, 143-145). One possible explanation for the association between high MMP levels and fibrosis is that MMPs may be mainly expressed outside of the interstitial compartment where collagen is accumulating (146). However, these proteinases may in fact promote a fibrotic response as a result of their multiple biological functions outside of collagenolysis including apoptosis, migration, proliferation and angiogenesis (147). Matrylisin (MMP-7), for example, regulates TGF-β activity via the release of pre-formed TGF-β from the extracellular matrix (147), and interacts with osteopontin, an inflammatory cytokine that promotes extracellular matrix deposition and induces growth and migration of fibroblasts and epithelial cells (148). Inhibition of MMPs therefore represents an attractive therapeutic target in IPF. Doxycyline, an MMP inhibitor (149), has been observed to attenuate fibrosis,

inhibiting MMPs, collagen-1, TGF-β, and CTGF in human type II AECs and bleomycin-exposed mice (150). Doxycycline was tested in two open-label studies performed in India, and a non-statistically significant trend toward improved 6mwt and FVC was observed (151,152). These studies were quite small (n=6 patients each), however, and doxycycline may merit further investigation in larger, controlled clinical trials.

Targeting the renin-angiotensin system

The renin-angiotensin system is a key regulator of blood pressure homeostasis. Renin, a protease, cleaves its only known substrate (angiotensinogen) to form angiotensin I, which in turn serves as substrate to angiotensin converting enzyme (ACE) to form ANGII. Renin and ANGII have both been implicated in IPF pathogenesis.

ANGII is a powerfully vasoactive hormone whose pleitropic effects are mediated by two receptors highly expressed in IPF lungs: angiotensin type 1 (AT1) and angiotensin type 2 (AT2) (153). ANGII induces apoptosis in alveolar epithelial cells (154) and pulmonary arterial endothelial cells (155), and the proliferation, activation, and migration of fibroblasts, resulting in abnormal deposition of ECM components (153). Myofibroblasts from IPF lungs synthesize more ANGII and active TGF-β than fibroblasts from normal lungs, with ANGII driving the production of this pro-fibrotic cytokine and resulting in increased myofibroblast differentiation in a process that has been described as an "angiotensin/TGF-β1 autocrine loop. (156)" Bleomycin-induced lung injury is attenuated by administration of ACE inhibitors (ramipril or captopril) (157), or an AT1 inhibitor (losartan) (158,159), or deletion of the AT1 gene (158). Inhibition of ANGII or its receptors thus represents an attractive target for the treatment of IPF, and the safety and efficacy of losartan are currently being investigated in a phase II open-label clinical trial of IPF (clinicaltrials.gov identifier NCT00879879).

It is unclear whether the use of ACE inhibitors is efficacious in human lung fibrosis, however, as a lack of observed benefit has been reported in some studies (160,161). ACE2, another regulator of the renin-angiotensin system that converts ANGII into its anti-apoptotic degradation product ANG1-7, is notably underexpressed in IPF lungs (162). ANG1-7 inhibits the activation of JNK, in effect regulating AEC survival (163), and the systemic administration of purified recombinant

ACE2 has been shown to reduce bleomycin-induced lung collagen deposition in mice (162). It has therefore been suggested that the excessive signaling by ANGII may be due to its impaired degradation and the loss of an inhibitory signal rather than to its increased synthesis, and that agents that enhance ANGII metabolism, such as ACE2, may thus be effective against lung fibrosis (164).

Renin has recently also been noted to be a pro-fibrotic mediator of lung fibrosis that functions independently from ANGII. Its effects in human IPF lungs and fibroblasts include a marked increase in TGF-β and collagen. Renin gene silencing results in the reduced expression of collagen and TGF-β1 in vitro. Renin inhibition could thus potentially ameliorate IPF fibrosis (165).

Other potential therapies for IPF
Carbon monoxide

The enzymatic product of heme oxygenase activity, carbon monoxide (CO), is a biologically active diatomic gas endogenous to healthy and diseased humans. CO has well-described anti-proliferative properties (166-168), and there is evidence that CO is protective in the setting of lung injury (169,170). Short, transient exposure to CO has also demonstrated to reduce fibrosis in the bleomycin model (171). It is thought that the antifibrotic effects of CO may be at least in part due to its inhibition of TGF-β-induced ECM constituents fibronectin and type I collagen production in fibroblasts (171). Futher, administration of quercitin, an inducer of heme oxygenase, results in the attenuation of TGF-β-stimulated collagen production in human fibroblasts (172). While the mechanisms driving the antifibrotic properties of CO have not yet been fully elucidated, low-dose inhaled CO is currently being tested as a potential IPF therapy in a phase II trial (clinicaltrials.gov identifier NCT01214187).

Adjunctive treatment of gastroesophageal reflux

The prevalence of gastroesophageal reflux (GER), symptomatic or "silent," has been estimated to be as high as 88% in IPF patients (173,174), prompting the hypothesis that injury to the lung tissue caused by repeated microaspiration triggers the development of fibrosis (175). While it is possible that GER may develop as a consequence of anatomical remodeling caused by progressive fibrosis, data from animal studies (176,177) and small human case series (178,179) support the role of GER as pathogenic for IPF. One recent observational study of 204 IPF patients from two centers revealed an association between GER medication use

[in the form of proton pump inhibitors (PPIs) or histamine-2 receptor (H2) blockers] and improved survival (HR=0.47), along with a decreased HRCT fibrosis score (14% compared to 19% in those not taking medications) (180). While these results suggest that GER therapy may be of benefit in IPF, further study is needed to demonstrate a causal relationship to improved survival.

Stem cell therapy

Restoration of the alveolar epithelium is of course the most desirable of therapeutic effects in the setting of IPF. When the lung is injured, there is an intense production of inflammatory signaling molecules to recruit progenitor cells and stem cells to the site of injury to restore the integrity of the epithelial layer and alveolar capillary units (181). In IPF, however, a premature exhaustion of the renewal potential of epithelial stem cells, possibly caused by telomere shortening in the setting of environmental insult (e.g., smoking, pollution), is one probable cause of the loss of epithelial integrity and abnormal alveolar re-epithelialization (182). Promisingly, pluripotent stem cells derived from embryonic or adult tissues can differentiate into lung epithelial and endothelial cells, ameliorating lung injury and fibrosis as demonstrated in several preclinical studies (181,183-188). Although it is not clear whether structural engraftment or a paracrine/immunomodulatory effect produced by the stem cells is responsible for these potentially therapeutic effects (189), this therapy could potentially result in the regeneration and repair of diseased adult lungs. One recent study of intravenous mesenchymal stem cell therapy to restore the myocardium after acute infarction revealed that a majority of these cells were sequestered by the lung, and this was associated with improvement in the pulmonary function of treated subjects (190). It is important to note, however, that pluripotent cells have been associated with spontaneous transformation and induction of malignancy, and it is also possible that their great plasticity could lead to differentiation into unwanted cell phenotypes with untoward effects (189,191). Nevertheless, a Phase I, open-label safety and feasibility study of mesenchymal stem cell treatment for IPF in up to 8 subjects was started in Australia (clinicaltrials.gov identifier NCT01385644). While the enrollment status or results of this trial are not yet published, the US FDA very recently approved the first clinical trial of intravenous mesenchymal stem cell therapy for IPF, a phase I study yet to be listed in clinicaltrials.gov.

Lung transplantation

At present, the only intervention that improves survival in select patients with IPF is lung transplantation. In a study of 46 patients awaiting lung transplantation, survival was increased by 79% one year post transplant, and the relative risk reduction for those who underwent lung transplantation was 75% (P=0.03) compared to patients who remained on the waiting list (192). Despite its success, lung transplantation is not without significant risks. The most common complications and causes for poor long-term survival after transplantation include infection (given the need for immunosuppression), acute and chronic graft rejection, and airway stenosis (193). The general age cut-off for lung transplantation is 65 years, while there are exceptions based on the patient's functional capacity and comorbidities (193). Of note, the adoption of the newer lung allocation score system has resulted in significant reduction in both wait times and mortality on the wait list for IPF patients (66). More recently, bilateral lung transplantation (BLT) has become preferred when compared to single lung transplantation (SLT). Data from the International Society for Heart and Lung Transplantation demonstrated that between January 2000 to June 2005, 1- and 5- year survival rates for SLT in IPF were 76% and 45% respectively (n=1,084), and for BLT were 77% and 52.5% respectively (n=687) (194).

The general guidelines for lung transplantation include a baseline carbon monoxide diffusing capacity (DLco) of less than 35% to 39% predicted, a desaturation during a 6MWT to less than 88%, and a decline in the FVC of 10% or greater when compared over a 6-12 month period of time (193). Nevertheless, guidelines for referral and listing for transplantation by the International Society for Heart and Lung Transplantation recommend referring patients with IPF (with histologic or radiographic evidence) for transplant evaluation early, regardless of the FVC parameters (193,195). In fact, data from a recent single-center prospective study reveal that a delay from onset of dyspnea until evaluation at a tertiary care center is associated with a higher rate of death from IPF independent of disease severity (196).

Clinical trials

Recently completed and ongoing studies are summarized in Tables 2 and 3, respectively. Over the past decade, the

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definition of IPF and thus enrollment criteria for this disease have become more specific, however, study design still remains a challenge as there is continued debate on what constitutes a clinically meaningful endpoint. While all-cause mortality and all-cause non-elective hospitalization have been proposed as the best choices (197), measuring these outcomes could be prohibitive, requiring the enrollment of a large number of patients to be followed over an extensive period of time. Others have proposed that the widely adopted primary endpoint of lung function, specifically FVC, is in fact clinically meaningful. Nevertheless, due to the dearth of therapeutic agents approved for the treatment of IPF, patients should be strongly encouraged to participate in randomized, multi-center, placebo-controlled trials (1,193). A registry of federally that privately supported clinical trials at "clinicaltrials.gov" lists active or recently completed studies of IPF and can be accessed by referring physicians (193).

Conclusions

Over the past 10 years, substantial advances have been made

in the understanding of the pathophysiology of IPF. As new pathogenic pathways and mediators are discovered, new therapies in development are more sharply focused on the fibroblastic process, sharing as their target abnormal tissue remodeling, excessive extracellular matrix accumulation, and angiogenesis, all believed to be at the heart of this progressive disease. While it is likely that any effective treatment strategy for IPF will need to target more than one of the pro-fibrotic pathways associated with its complex pathogenesis, only one therapeutic agent has been approved to date worldwide. The development of new treatment modalities is therefore critically important. Although the mechanisms underlying this disease remain poorly understood, the advances that have been made to date provide us with hope for the discovery and development of effective treatment modalities in the near future.

Acknowledgements

Table 2 An overview of select clinical trials of in IPF - Unsuccessful Treatments to Date. (Modified from Datta et al., 2011)

Agent/treatment	Potential mechanism of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Corticosteroids	Suppression of cellular and humoral immunity; reduction of proinflammatory molecules	Significant lack of studies evaluating prednisolone against placebo Flaherty et al. (2001)	None available	Open label study; (n=41)	Primary end point: CRP score at 3 months	27% responders/ 46% stable/ 27% non-responders. Adverse effects noted in all patients Cochrane Review of 2003 found no evidence for an effect of corticosteroids in IPF; no high quality prospective studies were identified as suitable for meta-analysis (Richeldi et al., 2003)
Azathioprine as adjunctive to prednisolone	Inhibits adenine deaminase and impairs cell proliferation (particularly leukocytes); anti-inflammatory	Raghu et al. (1991)	None available	Prospective, double-blinded, randomized placebo-controlled trial; prednisolone + azathioprine (n=14) vs. prednisolone + placebo (n=13)	Primary end points: $\Delta FVC/DL_{CO}/A$-a gradient at 1 year; survival at 9 years	Marginally significant survival benefit in azathioprine + prednisolone group only after age-adjustment No significant improvement in remaining parameters
Azathioprine + prednisolone	As above	Thorax National Institute, Chile	NCT00518310	Prospective, double-blinded, randomized trial; Azathioprine + prednisolone vs. placebo; planned enrollment (n=100)	Primary end point: progression free survival at 2 years	Trial status unknown; results awaited
Cyclophosphamide	Alkylating agent with anti-inflammatory properties	Collard et al. (2004)	None available	Retrospective case series; cyclophosphamide + prednisolone vs. no treatment; (n=82) in each group	Primary end point: Survival at 6-12 months	No evidence for a therapeutic benefit. Significant potential adverse effects
Everolimus	Immunosuppressant-macrocyclic proliferation cyclic inhibitor	Malouf (2011)	ANZCTR 12605000599673	Prospective, double-blinded, randomized placebo-controlled trial; everolimus (n=44) vs. placebo (n=45)	Primary end point: Δ6MWD, arterial oxygen saturation, quality of life, and dysnpea score up to 36 months	Trial completed; increased time to disease progression in treatment group. 180 days vs. 450 days for placebo group. 48% of patients in treatment arm did not tolerate an 8mg dose. 23% of these patients discontinued for this reason

Table 2 (*continued*)

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Table 2 (*continued*)

Agent/treatment	Potential mechanism of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Warfarin	Anticoagulation via inhibition of Vitamin K reduction	Kubo et al. (2005)	None available	Randomized open label trial; prednisolone + warfarin/low molecular weight heparin(n=31) vs. prednisolone + placebo (n=33)	Primary end points: time to death and hospitalization-free time over 1 year	Anti-coagulant therapy resulted in a significant increase in survival of patients with IPF and a significant improvement in survival associated with acute exacerbations of IPF
Warfarin	As above	ACE-IPF trial NHLBI – Duke University, USA Noth et al. (2012)	NCT00957242	Prospective, double-blinded, randomized placebo-controlled trial; warfarin vs. placebo; currently recruiting, planned enrollment (n=256)	Primary end points: time to death or disease progression over 48 weeks	Trial terminated; excess mortality in warfarin arm (14 warfarin vs. 3 placebo deaths). Low probability of treatment benefit. Higher rates of hospitalization and acute exacerbation.
Heparin	Anticoagulation via inhibition of thrombin and other proteases.	Markart et al. (2010)	None available	Open label exploratory study evaluating safety of nebulized heparin in IPF; (n=21)	Study designed to assess safety and tolerability	Trial completed; adequate local anticoagulation achieved with no significant adverse effects. Future trials planned to evaluate efficacy.
Bosentan	Endothelin-1 (ET) receptor antagonist; ET promotes fibroblast proliferation, differentiation, collagen synthesis, and endothelial cell mitosis	BUILD-1 trial King et al. (2008)	NCT00071461	Prospective, double-blinded, randomized placebo-controlled trial; bosentan (n=74) vs. placebo (n=84)	Primary end point: 6MWD at 12 months	Trial completed; no effect on primary outcome between treatments arms; post hoc-canalysis demonstrated trend in delayed time to disease progression or death in the bosentan arm of IPF patients who had undergone lung biopsy
Bosentan	As above	BUILD-3 trial (Actelion, Switzerland)	NCT00631475	Prospective, double-blinded, randomized placebo-controlled trial; total (n=616), bosentan 2:1 recruitment complete	Primary end points: time to disease progression or death over 8-32 months	Trial terminated at interim analysis stage due to lack of efficacy

Table 2 (*continued*)

Table 2 (*continued*)

Agent/treatment	Potential mechanism of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Ambrisentan	As above	ARTEMIS-IPF trial (Gilead, USA)	NCT00768300	Prospective, double-blinded randomized placebo-controlled trial; ambrisentan vs. placebo, currently recruiting, planned enrollment (n=600)	Primary end points: time to disease progression or death, event driven over 4 years	Trial terminated at interim analysis stage due to lack of efficacy
Macitentan	As above	MUSIC trial (Actelion, Switzerland)	NCT00903331	Prospective, double-blinded randomized placebo-controlled trial; total n=178; macicentan vs. placebo, recruitment complete	Primary end point: ΔFVC over 12 months	Trial terminated; did not meet primary endpoint between treatment arms of FVC
Sildenafil	Phosphodiesterase 5 inhibitor. Causes vasorelaxation by stabilizing cGMP	Step-IPF Clinical Research Network, USA (Zisman et al., 2010)	NCT00359736	Prospective, double-blinded, randomized placebo-controlled trial :sildenafil (n=89) vs. placebo (n=91). Double-blind study over initial 12 weeks, followed by open label extension for 12 weeks with all patients receiving sildenafil	Primary end points: Δ 6MWD over 12 weeks Secondary end point: dyspnea score at 6 months	Trial completed; No significant improvement in primary end point in treatment arm, but significant improvement in secondary end points in sildenafil arm, including DL_{co} and quality of life score
Interferon (IFN γ 1b)	Immunoregulatory cytokine limiting fibroblast proliferation and collagen synthesis	INSPIRE trial King et al. (2009)	NCT00075998	Prospective, double-blinded, randomized placebo-controlled trial; interferon (n=551) vs. placebo (n=275)	Primary end point: survival from time of randomization	Trial ended prematurely; overall survival had crossed predefined boundary at planned interim stage analysis (64 weeks); however, no difference between treatment and placebo arms

Table 2 (*continued*)

Table 2 (*continued*)

Agent/treatment	Potential mechanism of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Inhaled IFN γ 1b	As above	National Centre for Research Resources, USA Diaz et al. (2012)	NCT00563212	Non-randomized, open-label, single interventional study with nebulized interferon-γ Recruiting patients; planned enrollment n=10	Primary end point: safety and tolerability Secondary end points: lung function trends and BALF [IFN-γ] at 1 year	Trial completed; aerosolized IFN γ 1b was well tolerated and associated with minimal change in FVC over 80 weeks and a decreased slope of decline in TLC and DlCO
Etanercept	TNF inhibitor -anti-inflammatory, anti-fibrogenic	Raghu et al. (2008)	NCT00063869	Prospective, double-blinded, randomized placebo-controlled trial; etanercept (n=34) vs. placebo (n=31)	Primary end points: Δ FVC, DL$_{co}$/ΔA-a gradient over 48 weeks	Trial completed; no significant difference observed between treatment groups. Etanercept therapy resulted in a non-significant reduction in disease progression in several physiological, functional and QoL end points
Imatinib mesylate	Inhibitor of PDFG and TGF β signaling, which promote fibroblast to myofibroblast transformation and proliferation and ECM production	Daniels et al. (2010)	NCT00131274	Prospective, double-blinded, randomized placebo-controlled trial; imatinib (n=59) vs. placebo (n=60)	Primary end point: time to disease progression (>10% decline in predicted FVC) or death over 92 weeks	Trial completed; no change in primary end point between treatment and placebo
CC-930	JNK inhibitor-JNK induces tissue factor expression and thrombin and fibren generation	Celgene Corporation	NCT01203943	Prospective, double-blinded, randomized placebo-controlled trial; planned enrollment n=28	Primary end point: safety up to 4 weeks of treatment Secondary end point: pharmacokinetics and long-term safety	Trial terminated at interim analysis stage due to unfavorable risk benefit profile.

6MWD, 6 min walk test distance; A-a, alveolar:arterial ANZCTR, Australian New Zeland clinical trials registry; BALF, bronchoalveolar lavage fluid; CCL-2, Chemokine (C-C motif) ligand 2; cGMP, cyclic guanosine monophosphate; CRP, clinical-radiographic-physiological; DLco, carbon monoxide dilution; FGFR, fibroblast growth factor receptor; FVC, forced vital capacity; H2, histamine H2 receptor blocker; HRCT, high resolution computer tomography; IFN-γ, interferon-gamma; IL-13, interleukin 13; IL-4, interleukin 4; LOXL-2, lysyl oxidase-like enzyme 2; MMP, matrix metalloproteinase; NCT, clinicaltrials.gov identifier; PDGFR, platelet-derived growth factor receptor; PPI, proton pump inhibitor; pred, predicted QoL, quality of life; TGF β, transforming growth factor-beta; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Permission has been obtained from John Wiley and Sons for reuse of figure Table 2.

Table 3 An overview of select clinical trials of in IPF - Treatments Under Investigation (Modified from Scotton and Chambers, 2007 and Datta and Scotton, 2011 BJP)

Agent/treatment	Potential mechanisms of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Azathioprine + Prednisolone with or without N-acetylcysteine (NAC)	Antioxidant, immuno-suppressant, anti-inflammatory	IFIGENIA trial Demedts et al. (2005)	NCT00639496	Prospective, double-blinded, randomized placebo-controlled trial; NAC + azathioprine + prednisolone (n=92) vs. placebo + azathioprine + prednisolone (n=90)	Primary end points: absolute ΔFVC and DL_{CO} at 12 months	Trial completed; reduction in FVC and DL_{CO} decline over 1 year in NAC arm, though no change in mortality
N-acetylcysteine (NAC) with or without Azathioprine + Prednisolone	Antioxidant, immuno-suppressant, anti-inflammatory	Panther-IPF trial NHLBI, USA Raghu et al. (2012)	NCT00650091	Prospective, double-blinded, randomized placebo-controlled trial; currently recruiting patients, planned enrollment n=390	Primary end point: ΔFVC at 60 weeks	Increased mortality observed in the triple therapy arm. Triple treatment arm stopped for safety. Subjects on NAC or placebo alone continue to be followed
Pirfenidone	Antifibrotic inhibitor of TGFβ, anti-inflammatory, antioxidant	Taniguchi et al. (2010)	None available	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone (n=108) vs. low dose pirfenidone (n=55) vs. placebo (n=104)	Primary end point: ΔFVC at 52 weeks	Significant reduction in FVC decline in high dose treatment arm. However, change in end point during trial, handling of missing data and absence of patient reported outcome means it is difficult to draw firm conclusions at this time
Pirfenidone	As above	CAPACITY 1 trial (Intermune, USA) Noble et al. (2011)	NCT00287729	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone (n=171) vs. placebo (n=173)	Primary end point: ΔFVC at 72 weeks	Trial completed; no significant difference in FVC decline between treatment groups
Pirfenidone	As above	CAPACITY 2 trial (Intermune, USA) Noble et al. (2011)	NCT00287716	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone (n= 174) vs. low dose pirfenidone (n= 87) vs. placebo (n= 174)	Primary end point: ΔFVC at 72 weeks	Trial completed; significant reduction in FVC decline in pirfenidone groups

Table 3 (*continued*)

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Table 3 (*continued*)

Agent/treatment	Potential mechanisms of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Pirfenidone	As above	ASCEND trial (Intermune, USA)	NCT01366209	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone *vs.* placebo; planned enrollment n=500	Primary end point: Δ%FVC at 52 weeks	Trial ongoing; results awaited
GC1008	Anti-TGF β 1, 2, and 3 antibody	Genzyme and Cambridge Antibody Technology, UK	NCT00125385	Non-randomized, open label, single group assignment Phase I study (n=25)	Primary end points: safety and tolerability. Secondary end points: potential clinical outcomes up to 3 years	Trial completed; results awaited
STX-100	Anti- v β 6 integrin	Stromedix, USA	NCT01371305	Phase I studies completed (Stromedix) – awarded orphan drug status (USA) and a Phase II study is ongoing; planned enrollment n=35	Primary end point: safety over 24 weeks	Phase I Trial completed; results awaited. Phase II Trial ongoing
FG-3019	Connective tissue growth factor inhibitor	Fibrogen, USA	NCT00074698	Open-label Phase I study completed (n=21)- awarded orphan drug status (USA); an open-label Phase II study is ongoing (n=84)	Phase II trial primary endpoint: safety at 45 weeks. Secondary endpoints: effect on extent of pulmonary fibrosis, pulmonary function and dyspnea	Phase I trial completed; FG-3019 is safe and well-tolerated. Future trials will assess therapeutic potential. Phase II trial ongoing
Octeotride	Somatostatin analogue	Institut National de la Santé Et de la Recherche Médicale, France	NCT00463983	Non-randomized open label single interventional study with octeotide; (n=25)	Monitoring of FVC; DL_{CO}; HRCT fibrosis score; 6MWD over 48 weeks	Trial completed; trend of decline in FVC and DlCO was lower in subjects treated with octeotride compared to historical, previously published data from other trials

Table 3 (*continued*)

Table 3 (*continued*)

Agent/treatment	Potential mechanisms of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/ available	Outcome/comments
CNTO 888	Anti-CCL2 antibody	Centocor, USA	NCT00786201	Prospective, double-blinded, randomized placebo-controlled Phase II trial; CNTO 888 ± usual therapy *vs.* placebo ± usual therapy; currently recruiting patients, planned total n=120	Primary end points: safety and performance at lung function tests	Trial completed; results awaited
QAX576	Anti-IL-13 antibody; IL-13 stimulates collagen deposition and myofibroblast differentiation	Novartis, Switzerland	NCT00532233	Open label Phase II study (n=50)	Primary end point: IL-13 serum levels Secondary end point: change in designated serum biomarkers over time with treatment for 4 weeks	Trial completed; results awaited
Tralokinumab	Anti-IL-13 antibody; IL-13 stimulates collagen deposition and myofibroblast differentiation	MedImmune LLC.	NCT01629667	Prospective, double-blinded, randomized placebo-controlled Phase II study; high dose tralokinumab *vs.* low dose tralokinumab *vs.* placebo, planned enrollment n=186	Primary end point: change from baseline in FVC at week 72 Secondary end point: safety	Trial ongoing
SAR156597	Bispecific Anti-IL-13 and IL-4 antibody; IL-13 stimulates collagen deposition and myofibroblast differentiation; IL-4 promotes fibroproliferation	Sanofi-Aventis	NCT01529853	Prospective, double-blinded, randomized placebo-controlled Phase II study; SAR156597 *vs.* placebo, planned enrollment n=24	Primary end point: safety and tolerability over 6 months Secondary end point: change in FVC, DlCO and dyspnea score from baseline	Trial ongoing

Table 3 (*continued*)

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Table 3 (*continued*)

Agent/treatment	Potential mechanisms of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Thalidomide	anti-angiogenic immunomodulatory anti-inflammatory inhibitor of TGF β -1 signalling and VEGF expression	Investigator led – John Hopkins University, USA	NCT00162760	Non-randomized open label single interventional study designed for patients who have failed or are unsuitable for immunosuppressive therapy; planned enrollment n=19	Primary end point: safety Secondary end points: Δlung function over 1 year	Trial completed; results awaited
GS-6624	Anti-LOXL2 antibody; this enzyme generates crosslinks fibrillar collagen to generate the scaffold on which fibroblasts grow	Gilead Sciences	NCT01362231	Randomized, double-blind, dose escalation study of GS-6624 vs. placebo; planned enrollment n=48.	Primary end point: safety and tolerability	Phase I trial completed; results awaited Phase II trial planned
BIBF 1120	Angiokinase inhibitor targeting proliferative growth factors in fibroblasts (FGFR, PDGFR, VEGFR)	TOMORROW trial Boehringer Ingelheim Pharmaceuticals, UK	NCT00514683	Prospective, double-blinded, randomized placebo-controlled Phase II study; BIBF1120 vs. placebo; total (n=400); recruitment complete	Primary end point: ΔFVC over 1 year Secondary end point: dyspnea score, survival	Trial completed; results awaited
BIBF 1120	As above	INPULSISTM-1 and INPULSISTM-2 trials Boehringer Ingelheim Pharmaceuticals, UK	NCT01335464 and NCT01335477	Prospective, double-blinded, randomized placebo-controlled Phase III studies; BIBF1120 vs. placebo; planned enrollment n= 515 and 551, respectively.	Primary end point: ΔFVC over 52 weeks	Trials ongoing
Minocycline	Broad spectrum tetracycline with anti-inflammatory and anti-angiogenic properties	Investigator led trial – University of California, USA	NCT00203697	Prospective, double-blinded, randomized placebo-controlled trial; patient numbers not disclosed	Primary end points: safety and efficacy	Trial status unknown; results awaited

Table 3 (*continued*)

Table 3 (*continued*)

Agent/treatment	Potential mechanisms of action	Select clinical trial or retrospective series	Clinical trials registry number	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Tetrathiomolybdate	Antiangiogenic	Investigator-led trial – University of Michigan, USA	NCT00189176	Non-randomized, open label, uncontrolled, single group assignment Phase I/II (n=20)	Primary end point: safety Secondary end points: Δlung function tests	Trial completed; results awaited
Doxycycline	MMP inhibitor; some MMPs drive cellular apoptosis, migration, proliferation, and angiogenesis	Indian Institute of Chemical Biology, India	None available	Non-randomized, open label, uncontrolled, single group assignment (n=6)	Primary end point: inhibition of MMP activity in the BALF at 6 months Secondary end points: ΔFVC, 6MWD, and dyspnea score	Trial completed; a non-statistical trend toward improved 6MWD and FVC
Losartan	Angiotensin II inhibitor	National Cancer Institute, USA	NCT00879879	Open label interventional study; recruiting patients; planned enrollment n=25	Primary end point: FVC response at 1 year	Trial status unknown; results awaited
Carbon Monoxide	Anti-proliferative diatomic gas, inhibitor of fibroblast ECM deposition	Brigham and Women's Hospital, USA	NCT01214187	Prospective, double-blinded randomized placebo-controlled trial; carbon monoxide *vs.* placebo, currently recruiting, planned enrollment n=60	Primary end point: Δserum baseline MMP7 level at 3 months	Trial ongoing
Adjunctive treatment of GER with PPIs or H2 receptor blockers	Gastroesophageal therapy and/or prophylaxis	Lee et al. (2011) Raghu et al. (2006)	None available	Retrospective case series; PPI or H2 blockers *vs.* no GER therapy; (n=204)	Primary end point: survival from time of IPF diagnosis	Decreased HRCT fibrosis score (14 *vs.* 19%) and improved survival (HR=0.47) in the GER therapy group.
Mesenchymal stem cells	Potential alveolar re-epithelialization	The Prince Charles Hospital, Australia	NCT01385644	Prospective, open-label trial; low dose mesenchymal stem cells (MSC) *vs.* high dose MSC; planned enrollment n=8	Primary end point: safety 6 months post treatment	Trial ongoing

6MWD, 6 min walk test distance; A-a, alveolar:arterial ANZCTR, Australian New Zeland clinical trials registry; BALF, bronchoalveolar lavage fluid; CCL-2, Chemokine (C-C motif) ligand 2; cGMP, cyclic guanosine monophosphate; CRP, clinical-radiographic-physiological; DLco, carbon monoxide dilution; FGFR, fibroblast growth factor receptor; FVC, forced vital capacity; H2, histamine H2 receptor blocker; HRCT, high resolution computer tomography; IFN-γ, interferon-gamma; IL-13, interleukin 13; IL-4, interleukin 4; LOXL-2, lysyl oxidase-like enzyme 2; MMP, matrix metalloproteinase; NCT, clinicaltrials.gov identifier; PDGFR, platelet-derived growth factor receptor; PPI, proton pump inhibitor; pred, predicted QoL, quality of life; TGFβ, transforming growth factor-beta; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Permission has been obtained from John Wiley and Sons for reuse of figure Table 3.

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Acute exacerbation of idiopathic pulmonary fibrosis—a review of current and novel pharmacotherapies

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Abstract: Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive form of lung disease of unknown etiology for which a paucity of therapies suggest benefit, and for which none have demonstrated improved survival. Acute exacerbation of IPF (AE-IPF) is defined as a sudden acceleration of the disease or an idiopathic acute injury superimposed on diseased lung that leads to a significant decline in lung function. An AE-IPF is associated with a mortality rate as high as 85% with mean survival periods of between 3 to 13 days. Under these circumstances, mechanical ventilation (MV) is controversial, unless used a as a bridge to lung transplantation. Judicious fluid management may be helpful. Pharmaceutical treatment regimens for AE-IPF include the use of high dose corticosteroids with or without immunosuppressive agents such as cyclosporine A (CsA), and broad spectrum antibiotics, despite the lack of convincing evidence demonstrating benefit. Newer research focuses on abnormal wound healing as a cause of fibrosis and preventing fibrosis itself through blocking growth factors and their downstream intra-cellular signaling pathways. Several novel pharmaceutical approaches are discussed.

Keywords: Idiopathic pulmonary fibrosis (IPF); acute exacerbation (AE); drug therapy; treatment; clinical trials

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive form of lung disease with an unknown etiology that occurs primarily in adults in their fifties and sixties (1). The annual incidence of IPF in the USA using narrow case definitions has been reported to range from 6.8-16.3 cases per 100,000 population and 0.22-7.4 cases per 100,000 population in Europe (2). The prognosis is poor, with older studies reporting a median survival rate of 2-3 years from the time of diagnosis (3-5). While some patients with IPF will experience a progressive decline in lung function over time (6,7), the clinical course can be highly variable. Some patients experience acute exacerbations of IPF (AE-IPF) resulting in sudden progression of the disease with an up to 85% mortality rate during or immediately after AE-IPF

(8,9). Treatment regimens for stable IPF in the past had mainly focused on decreasing inflammation, particularly with high dose steroids, to prevent progression to fibrosis. However, most anti-inflammatory therapies including corticosteroids have shown no significant benefits (6,10,11). Newer research has focused on abnormal wound healing mechanisms as a cause of fibrosis; raising the prevention of fibrosis itself as therapeutic target through the blocking of growth factors and downstream intra-cellular signaling pathways (12-14).

Disease-modifying treatment of IPF has awakened with the publication of results of several phase-3 clinical trials (15-17). King *et al.* (15) reported on the use of pirfenidone, an oral antifibrotic drug, in the Assessment of pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis (ASCEND) trial and found that it met its primary

end point of a significant reduction in the one-year rate of decline in forced vital capacity (FVC). However, this trial did not assess effective treatment of AE-IPF. Indeed, one of the adverse events that led to discontinuation of study treatment was a worsening of IPF (15). Richeldi *et al.* (16) studied the effects of nintedanib, an inhibitor of several tyrosine kinases, in the INPULSIS-1 and -2 trials. While nintedanib reduced the decline in FVC in IPF patients, its effect was not consistent when applied to the risk of investigator-reported AE-IPF in these trials (16). The IPF Clinical Research Network evaluated the utility of N-acetylcysteine (NAC) as an IPF therapy in the Prednisone, Azathioprine, and NAC: A Study That Evaluates Response in Idiopathic Pulmonary Fibrosis (PANTHER-IPF) trial, and found that NAC had no positive effect on its primary endpoint of change in FVC after 60 weeks of treatment, or on the frequency of IPF exacerbations as compared to placebo (17). None of these trials reported an improved survival.

Understanding and controlling exacerbations represents a challenge. Given the significant mortality associated with AE-IPF (1,6,7,9,18), research into the pathogenesis, diagnosis and treatment of these exacerbations is imperative. This review highlights current and novel pharmacological therapeutic management approaches for AE-IPF.

Acute exacerbations of IPF (AE-IPF)

An AE-IPF is a sudden acceleration of the disease or an acute injury superimposed on already diseased lung (8,9). This process must be distinguished from other acute events such as infection, pulmonary embolism, pneumothorax, and heart failure, all of which can present in a very similar fashion to that of an AE in patients with IPF (8,9). There is no universal definition of an AE-IPF, but standard criteria proposed by Collard *et al.* (9) help to distinguish AE from conditions that may mimic its presentation. These criteria include a previous or concurrent diagnosis of IPF with unexplained worsening or development of dyspnea within 30 days and new bilateral ground-glass abnormality and/or consolidation superimposed on a background reticular or honeycomb pattern consistent with usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT). The final requirement is the exclusion of other etiologies including infection, left-sided heart failure, pulmonary embolism, and an identifiable cause of acute lung injury (9).

Epidemiology

Incidence

The incidence of AE-IPF is highly variable in previous studies due to differences in study design as well as the lack of a standard definition for AE. A retrospective review of 461 patients with IPF using the definition of AE-IPF proposed by Collard *et al.* (9) reported a 1- and 3-year incidence of 14.2% and 20.7%, respectively (8). Another retrospective study utilizing the criteria for AE-IPF proposed by Taniguchi *et al.* in 2004 (19) involving 74 patients revealed a 1- and 3-year incidence of 8.6% and 23.9%, respectively (20). Kishaba *et al.*, (21) using a slightly broader definition of AE, which included a sudden aggravation of dyspnea within 30 days associated with new bilateral infiltrates in patients with known IPF, found an incidence of AEs of 9.8% in a cohort of 594 IPF patients over a 10-year period. The time to first incidence of AE-IPF is also highly variable with reports ranging from 3 to 60 months after initial clinic visit (5).

Risk factors and survival

Risk factors for AE-IPF identified by Song *et al.* (8) include lower FVC at baseline (mean of 72.0% of predicted for AE-IPF patients *vs.* 77.6% of predicted for patients without rapid deterioration at initial diagnosis; HR 0.979, P=0.011) as well as never having smoked (HR 0.585, P=0.050). The prevalence of baseline pulmonary hypertension (PH) in patients with IPF is also high, ranging from 32-46% in previous studies (22-25). PH at baseline is also associated with a significant risk of a subsequent AE-IPF (HR 2.217, P=0.041) and the presence of PH is associated with poorer overall survival (HR 4.74, P=0.206) (26). While the incidence of AE in relation to disease severity is unclear, more extensive disease on chest HRCT, including traction bronchiectasis, honeycombing, ground glass opacity and consolidation, is associated with higher mortality in AE-IPF, with a reported 3-month mortality of 80.6% in patients with extensive HRCT disease-stage compared to 54.5% in patients with limited disease-stage (P=0.007) (21). A systematic review of studies reporting 1- and 3-month survival rates after AE-IPF, demonstrated a pooled mortality rate over eight studies of 60% and 67%, respectively (27). Outcomes after AE with rapid deterioration in IPF are also poor, with a reported median survival of only 2.2 months from onset (8). Other smaller AE case series reported mortality rates as high as 85% and mean survival periods of

only 3-13 days (3,28-32). However, again, the definition of AE was not uniform in many of these older studies (33).

Etiology

The cause of AE-IPF is unknown. It is possible that AE may constitute a sudden acceleration of the underlying fibrotic disease (as characterized by enhanced epithelial injury and proliferation, and coagulation abnormalities) (34), or it may be triggered by other processes (35). Several possible mechanisms are proposed, including unrecognized infectious etiologies (6,36), and diffuse alveolar damage (DAD) caused by gastroesophageal reflux (GER) -related microaspiration (37-39) and medications such as α-interferon (40).

AE-IPF is associated with invasive procedures. Several case reports point to surgical lung biopsy, lung cancer resection, and bronchoscopy as causes of AE-IPF (41-43). A case series by Ghatol et al. suggested that AE-IPF can occur after either pulmonary or non-pulmonary surgery, with one patient experiencing AE-IPF following a total knee replacement (44). The authors postulate that intraoperative exposure to high oxygen concentrations and the high airway pressures or tidal volumes associated with mechanical ventilation (MV) may have been the precipitating factors. During the postoperative period, the parenchymal lung injury resulting from hyperoxia, barotrauma, or volutrauma could enhance the recruitment of circulating fibrocytes to the lung, resulting in worsening fibrosis (44). Although this suggests an association between AE-IPF and surgery, a causal relationship has not been proven.

Because patients with AE-IPF often present with symptoms suggestive of respiratory viral infection, viruses have also been considered a potential cause of AE-IPF. Herpesvirus and transfusion transmitted virus have been found in association with IPF and AE-IPF (45,46); an AE-IPF has also been reported after pandemic influenza A (H1N1) vaccination (47). It has thus been proposed that a considerable proportion of events deemed to be AE-IPF, are likely due to sequelae of infection (48). However, a well-controlled study by Wootton et al. found evidence of viral respiratory infection in only 4 of 34 patients who presented with AE-IPF (49). It would appear that viral infections do not play a major role in AE-IPF, however, findings in that study may be limited by the fact that patients may have presented after the initial infection could be detected, as such, viruses cannot be definitely excluded as the cause of AE-IPF (50).

Imaging and histopathology

The most common radiological finding in patients with AE-IPF is new ground-glass opacities superimposed on subpleural reticular and honeycombing densities (51). The pattern of ground glass opacities can be variable in degree and can range in their distribution from peripheral to multifocal to diffuse. Diffuse and multifocal ground glass patterns appear to predict a worse survival in patients with AE-IPF compared with patients peripheral patterns (28,52). In one series, median survival was reported as 16, 240, and 540 days for patients with diffuse, multifocal, and peripheral patterns on HRCT, respectively (OR =4.629; P=0.001 for combined diffuse and multifocal versus peripheral patterns) (52). The histopathology of AE is most commonly described as DAD superimposed on underlying UIP (6,9). It has also been characterized as UIP with organizing pneumonia or other findings not consistent with DAD, including acute lung injury without hyaline membrane formation. Wide distribution of fibroblastic foci away from the established DAD has also been observed (30,36,53).

Treatment of AE of IPF

Consensus guidelines

To date, no blinded, randomized, controlled trials specifically directed at the treatment of AE-IPF have been reported. The management approach currently recommended by international consensus, the American Thoracic Society, the European Respiratory Society, the Japanese Respiratory Society, and the Latin American Thoracic Association (ATS/ERS/JRS/ALAT) guidelines, includes only the use of supportive care and corticosteroids. Specifically, the recommendation is, "corticosteroids should be used in the majority of patients with AE-IPF, but not using corticosteroids may be a reasonable choice in a minority". This recommendation is weak and based on very low quality evidence, and no specific recommendations regarding the dose, route, and duration of corticosteroid therapy are made. There is consensus that supportive care should be the mainstay of therapy for AE-IPF (1). French practical guidelines for the treatment of AE-IPF state that in addition to their recommendation for the use of supportive care and corticosteroids, "it is possible to use intravenous cyclophosphamide". They also mention that there are "insufficient data regarding the use of low-molecular weight heparin to treat AE of IPF", but that "anticoagulant therapy may be prescribed in the case of

acute worsening of symptoms or if thromboembolic venous disease is suspected", and that "wide-spectrum antibiotics may be used when infection has not been definitely ruled out" (54).

The efficacy of these management strategies is largely untested and mortality continues to be high (8,9). Newer strategies targeting inflammatory mediators, fibrogenic mediators, fibroblast proliferation, and autoimmunity have also been preliminarily examined, and further studies in these areas are ongoing. Approaches for the treatment and prevention of AE-IPF are summarized in *Tables 1* and *2*, respectively.

Corticosteroids

Acute respiratory distress syndrome (ARDS) is typically characterized by DAD, as is AE-IPF. Because the potent anti-inflammatory properties of corticosteroids may offer some benefit in some stages of ARDS (79-81), corticosteroid therapy is often selected to treat AE-IPF despite the lack of clear evidence to support its use for this indication (1,82). Some have reported adverse outcomes in association with corticosteroid use in AE-IPF, and cautioned that support for their use may be unjustified on the grounds that the same evidence used to recommend against corticosteroids in stable IPF, is used to recommend for them in AE-IPF in the absence of a known difference in their pathogenic mechanisms (83,84). Initial reports on the use of corticosteroids in AE-IPF described varying degrees of improvement in chest X-ray findings, pulmonary function, and blood gas values with high dose treatment (1,000 mg daily of methylprednisolone sodium succinate followed by taper) (36). Subsequent retrospective reviews of larger cohorts of patients treated for AE-IPF with corticosteroids in addition to other therapies lacked a uniform definition of AE and showed very high mortality rates. Specifically, for patients treated with steroids as sole immunosuppressive therapy, reported in-hospital mortality was 55% in 65 patients who received methylprednisolone pulse ≥500 mg/day or prednisolone in high (≥0.5 mg/kg) or low doses (≤0.5 mg/kg) (8); 100% in 14 patients in a review in which corticosteroid type and dose was not reported (55); and, 3-month mortality was 82% in 11 patients treated with corticosteroids and corticosteroid pulses (methylprednisolone 1 g/day for 3 days) (6).

Corticosteroids do not appear to prevent AE or reduce overall mortality. A recent meta-analysis of the placebo arms of randomized controlled trials of IPF by Atkins *et al.*

demonstrated no statistical difference in mortality between trials that permitted (n=5) or disallowed (n=1) low dose corticosteroid use (11). In fact, the incidence of AE-IPF was lower in a study not permitting immunosuppressants than in those studies allowing corticosteroid therapy, and there were significantly more lower respiratory tract infections in patients receiving corticosteroids. Individual data regarding the dose and time course of corticosteroid use was not presented in this meta-analysis.

Corticosteroids and immunosuppression

Patients with AE-IPF may have elevated erythrocyte sedimentation rates or C-reactive protein (CRP) levels and leukocytosis in the absence of infectious agents. This, along with increased inteleukin-8, α-defensin, and ST2 protein levels (agents involved in fibrocyte recruitment or proliferation), suggests a triggered immune response with an active inflammatory environment rich in activated T cells and neutrophils (9,55,56). Several groups have combined the use of corticosteroids with cytotoxic agents such as cyclosporine or cyclophosphamide, and reported better survival in patients treated with the combination (8,56,58,60). While these studies have been largely retrospective and included small numbers of subjects under various definitions of AE-IPF, this suggests that the use of immunosuppressants in conjunction with corticosteroids is more effective than corticosteroid monotherapy.

Tacrolimus and corticosteroids

Tacrolimus is an immunosuppressant used mainly in allotransplantation and collagen vascular disease. Tacrolimus binds to and inhibits the protein phosphatase calcineurin, preventing T cell activation and down regulating interleukin-2 (IL-2) and other cytokines associated with T helper lymphocytes, although it has also been observed to enhance lung injury during the acute inflammatory phase (85). Tacrolimus is also known to prevent immune activation by inhibiting nuclear factor κ-light-chain-enhancer of activated B cells-κB (NF-κB), and to inhibit TGF-β-induced collagen deposition (85,86). Its utility in AE-IPF was addressed in a retrospective review by Horita *et al.* (57). Of 15 patients who received corticosteroids and broad-spectrum antibiotics, 5 also received continuous infusions of tacrolimus for 5-14 days, followed by oral tacrolimus. Target blood levels were 20 and 5 ng/mL for infusion and oral regimes, respectively. Survivors were continued on tacrolimus after

Table 1 Overview of select agents of potential therapeutic utility in AE-IPF

Agents for treatment of AE-IPF*	Potential mechanisms of action	Selected study or retrospective series	Treatment course and dose	Study design and number of AE-IPF subjects	Outcome
Corticosteroids without cytotoxic agents	Suppression of cellular and humoral immunity; reduction of proinflammtory molecules and suppression of neutrophil and leukocyte migration into the lung	Kondoh [1993] [36] Tajima [2003] (55) Parambil [2005] (30) Kim [2006] (6) Sakamoto [2010] (56) Song [2011] (8) Horita [2011] (57) Inase [2003] (58)	Typically, IV MP pulse 500–1,000 mg/day followed by PN in high (≥0.5 mg/kg) or low doses (≤0.5 mg/kg)	Retrospective case reports involving a total of 125 patients treated with corticosteroids (n=3, 14, 5, 11, 11, 65, 10, and 6 patients per series). No controlled trials of corticosteroids for AE-IPF specifically have been reported.	Kondoh: Survival ranged from 5 months to 2.5 years after AE-IPF in 3 patients. In-hospital survival in subsequent series involving 84 patients ranged from 0% to 45%: Tajima: 14 of 14 patients died 5 to 274 days post admission Parambil: 5 of 5 patients died 20 to 57 days post admission Song: 36 of 65 patients died after an unspecified in-hospital follow-up period. 3-month survival in remaining 38 patients: Kim: 18% Sakamoto: 55% Horita: 20% Inase: 33%
Cyclophosphamide + corticosteroids	Cytotoxic alkylating agent with anti-inflammatory properties	Ambrosini [2003] (29) Parambil [2005] (30) Okamoto [2006] (59) Morawiec [2011] (60)	Ambrosini: IV MP pulses (500–1,000 mg/day for 3 days and then 125 mg/day) and CYP bolus (500 mg/m²) every 3 weeks Parambil: IV MP pulses 500–1,000 mg/day 3 days followed by PN, CYP 600–750 mg/m² every 3 weeks Okamoto: IV MP + CYP Morawiec: 1,000 mg MP pulses on days 1-3 followed by CYP infusion (500 mg) on day 4, increasing by 200 mg every 2 weeks up to 1,500 mg	Retrospective case reports involving a total of 45 AE-IPF (n=5, 2, 28, and 10 patients per series), and 7 SAE-IPF patients	Ambrosini: 1 of 5 patients survived at least 1.5 years until time of review; 4 of 5 died within 1 month. Survival: 1-month 20%, 3-month 20% Parambil: 2 of 2 patients treated after biopsy In-hospital survival: 0% Okamoto: 24 of 28 pts died within 30 days after AE-IPF with cyclophosphamide or cyclosporine A+ corticosteroids (report did not specify whether surviors received cyclophosphamide or cyclosporine A) Survival: 1-month 14%, 3-month 14% Morawiec: 2 of 10 AE-IPF patients 3 of 7 SAE-IPF survived 1 year. Overall 3-month survival was 72%. Survival in AE-IPF: 1-month: 100%, 3-month: 50%, 6-month: 40% Survival in SAE-IPF: 1-month: 100%, 3-month: 100%, 6-month: 71%

Table 1 (*continued*)

Table 1 (*continued*)

Agents for treatment of AE-IPF*	Potential mechanisms of action	Selected study or retrospective series	Treatment course and dose	Study design and number of AE-IPF subjects	Outcome
Tacrolimus + Corticosteroids	Inhibitor of calceinurin, NFκB, and TGF-beta-induced collagen deposition	Horita [2011] [57]	Tacrolimus group: continuous IV tacrolimus (target blood level 20 ng/mL) for 5-14 days, followed by oral tacrolimus (target blood level 5 ng/mL), continued after discharge + IV MP pulses (1,000 mg/day for 3 days, tapered to 80 mg/day over 8 days), followed by oral PN (1 mg/kg/day, tapered by 20%/week) Non-tacrolimus comparison group: MP pulses (1,000 mg/day for 3 days) followed by IV or oral glucocorticoid (1 mg/kg/day, tapered by 10 or 20%/week)	Retrospective review of 5 AE-IPF patients treated with tacrolimus and 10 AE-IPF patients not treated with tacrolimus	4 of 5 patients in the tacrolimus group and 1 of 10 patients in the non-tacrolimus group survived their acute exacerbations over the period in review. Survival in tacrolimus (n=5) vs. non-tacrolimus group n=10): 1-month 80% vs. 60% 3-month 80% vs. 20% Median survival time: >92 days for tacrolimus group 38 days for non-tacrolimus group Repeat exacerbation within 6 months: 0 of 4 surviving patients from the tacrolimus group 4 of 10 non-tacrolimus group patients
Cyclosporine A + Corticosteroids	Cytotoxic alkylating agent with anti-inflammatory properties; inhibits calceinurin and TGF-beta-induced collagen deposition	Okamoto [2006] [59] Homma [2005] [61] Sakamoto [2010] [56] Inase [2003] [58]	Okamoto: IV CsA + MP Homma: corticosteroids with or without IV CsA (50-200 mg/day, blood trough: 100-150 ng/mL) Sakamoto: MP pulse (1,000 mg/day 3 days) followed by PN (0.5-1.0 mg/kg, tapered within 4 weeks) with or without CsA (100-150 mg/day) Inase: MP pulse (1,000 mg/day for 3 days) followed by oral PN (40-60 mg/day taper) with or without CsA (1-2 mg/kg/day)	Retrospective case series involving a total of 55 AE-IPF patients (n=28, 9, 11, and 7 patients per series)	Okamoto: 24 of 28 pts died within 30 days after AE-IPF with cyclophosphamide or cyclosporine A+ corticosteroids (available report did not specify whether surviors received cyclophosphamide or cyclosporine A). Survival: 1-month: 14%, 3-month—14% Homma: Mean survival time for CsA (n=7) vs. non-CSA group (n=6): 9.9 vs. 1.7 months Sakamoto: Survival in CsA (n=11) vs. non-CSA group (n=11): 1-month 91% vs. 82% (approx.) 3-month 80% vs. 55% (approx.) Inase: Survival in CsA (n=7) vs. non-CSA group (n=6): 1-month 100% vs. 83% 3-month 71% vs. 33%

Table 1 (*continued*)

Table 1 (*continued*)

Agents for treatment of AE-IPF*	Potential mechanisms of action	Selected study or retrospective series	Treatment course and dose	Study design and number of AE-IPF subjects	Outcome
Sivelestat + Corticosteroids	Inhibition of neutrophil elastase	Nakamura [2007] (13)	Sivelestat and MP† therapy for 3 days, followed by prednisone (0.5 mg/kg/day)	Retrospective review of 10 patients treated with sivelestat	6-month survival: 40%
Azathioprine + Corticosteroids	Cytotoxic agent inhibiting leading to selective lymphocyte dysfunction. Suppresses natural killer cell activity, antibody production, and antibody-dependent cellular cytotoxicity	Shishido [1992] (62)	AZA (15 mg/kg) + MP pulse followed by PN (0.8 mg/kg)	Single AE-IPF case report	Patient survived >6 months after treatment. Tapering of immunosuppression led to a recurrence 3 months later, calling into question the diagnosis of IPF
Procalcitonin-guided antibiotic use	Tool for detecting of bacterial inflammation to guide initiation and discontinuation of antibiotics	Ding [2013] (63)	PCT level threshold of 0.25 ng/mL	Randomized controlled trial of PCT-guided antibiotic use (n=33 vs. routine antibiotic therapy (n=35)	PCT group vs. control group: Mean antibiotic treatment duation: 8.76.6 vs. 14.25.2 days Number of patients exposed to antibiotics: 79% vs. 100% No difference in outcome with respect to: Treatment success, mortality rate, days of hospitalization and duration of mechanical ventilation
Thrombomodulin + Corticosteroids	Anticoagulation by increased activation of protein C	Tsushima [2014] (64)	MP pulse (1,000 mg/day 3 days) followed by PN (1 mg/kg/day) + thrombomodulin (0.06 mg/kg/day 6 days)	Prospective open-label study using retrospective cohort of patients not treated with thrombomodulin for comparison	Thrombomodulin group vs. historical control group 28-day survival: 65% vs. 17%, P=0.048

Table 1 (*continued*)

Table 1 (*continued*)

Agents for treatment of AE-IPF*	Potential mechanisms of action	Selected study or retrospective series	Treatment course and dose	Study design and number of AE-IPF subjects	Outcome
Hemoperfusion with polymixin B-immobilized fiber column	Removal of endotoxins, reactive oxygen species, activated neutrophils, and proinflammatory, proangiogenic, and profibrotic cytokines (IL-9, IL-12, IL-17, PDGF and VEGF), amongst other substances	Seo [2006] (65); Noma [2007] (66); Tachibana [2011] (67); Abe [2012] (68); Oishi [2013] (69)	Seo: PMX-DHP (80-100 mL/min) 1-5 times for 2-6 hours over a variable time period, in combination with PN, CYP, or NAC. Noma: PMX-DHP over 3 days + MP pulse (1,000 mg/day 3 days) followed by CYP (500 mg/day) followed by PN (30 mg/day and AZA (150 mg/day) maintenance. Tachibana: PMX-DHP (80-100 mL/min) + high-dose corticosteroids. Abe: PMX-DHP (80-100 mL/min) once per day for 2 successive days + high-dose corticosteroids. Oishi: MP pulse (1,000 mg/day 3 days) followed + PMX-DHP (80-100 mL/min) over 2 successive days	Seo: open label pilot (n=6). Noma: case report (n=1). Tachibana: retrospective review (n=19). Abe: retrospective multi-center review (n=73). Oishi: prospective case series (n=9)	Seo: 1-month survival: 67%; Noma: survived until discharge 44 days after treatment. Tachibana: 1-month survival: 47.4%; 3-month survival: 26.3%. Abe: 1-month survival: 70.1%, 3-month survival: 34.4%. Oishi: 3-month survival: 66.7%. Typically, respiratory status and P/F ratio improved after first treatment, although not always sustained; cytokine and activated neutrophil levels in BALF decreased after therapy
Plasma exchange + rituximab + corticosteroids	Autoantibody removal by plasma exchange (PEX). Immunoglobulin load reduction through B-cell depletion by rituximab	Donahoe [2013—abstract] (70)	MP (1,000 mg/day 1 day, then tapered from 40 mg/day over 4 weeks) + 5 PEX (1.5 plasma volume) over 6 days followed by IV rituximab (1,000 mg once per week for 2 weeks)	Two open-label studies are being conducted (NCT01266317 and NCT01524068). The results of one open-label study of 6 patients have been published as an abstract	4 of 6 patients died within 38 days; 2 of 6 patients survived >60 days. 2 deaths were not directly attributable to AE-IPF These findings have not been peer-reviewed

*Therapeutic agents in this table were usually administered in combination with antibiotics and various supportive care measures. AE-IPF, acute exacerbation of IPF; AZA, azathioprine; BALF, bronchoalveolar lavage fluid; CsA, cyclosporine A; CYP, cyclophosphamide; IL, interleukin; IV, intravenous; MP, methylprednisolone; NAC, N-acetylcysteine; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PCT, Procalcitonin-guided antibiotic use; PDGF, platelet-derived growth factor; PEX, Plasma exchange; P/F, arterial oxygen tension (PaO₂)/inspiratory oxygen fraction (FiO₂); PMX-DHP, Hemoperfusion with polymixin B-immobilized fiber column; PN, prednisolone; SAE-IPF, sub-acute exacerbation of IPF; TGF-beta, transforming growth factor beta; VEGF, vascular endothelial grow.

Table 2 Overview of select agents of potential therapeutic utility for the prevention of AE-IPF

Agents for prevention of AE-IPF	Potential mechanisms of action	Selected study or retrospective series	Dose	Study design and number of subjects	Outcome
Corticosteroids	Suppression of cellular and humoral immunity; reduction of proinflammtory molecules and suppression of neutrophil and leukocyte migration into the lung	Atkins [2014] (11)	Typically, PN in low doses (≤20 mg/day)	Meta-analysis of placebo arms of eight randomized, controlled trials of various therapies in stable IPF; six trials reported on AE-IPF events Total patients in placebo arm n=1,631, total patients taking corticosteroids n=266	No difference in the rate of AE-IPF in trials permitting corticosteroid use *vs.* trials not allowing immunosuppression (IRR 3.93, P=0.14). Higher rate of AE-IPF in trials including patients with severe disease (IRR =0.23, P<0.0001); higher rate of use of low-dose immunosuppression in this group use may increase risk of AE-IPF. Incidence of respiratory infection was significantly higher in patients on corticosteroids (IRR 3.58, P<0.0001)
Azathioprine + Corticosteroids + N-acetylcysteine	Cytotoxic agent inhibiting leading to selective lymphocyte dysfunction. Suppresses natural killer cell activity, antibody production, and antibody–dependent cellular cytotoxicity	IPF Net [2012] (71)	Prednisone (0.5-0.15 mg/kg/day) + AZA (1.0-2.0 mg/kg/day) + NAC (600 mg 3 per day)	Prospective, double-blinded, randomized placebo-controlled trial of triple therapy (n=77) *vs.* NAC (n=81) *vs.* placebo (n=78) in stable IPF	Triple therapy arm was stopped early, after interim analysis. Triple therapy group *vs.* placebo group: Deaths: 8 *vs.* 1 (mostly due to respiratory causes) Hospitalizations: 23 *vs.* 7 AE-IPF events: 5 *vs.* 0 Serious adverse events: 24 *vs.* 0
Systemic anticoagulants + corticosteroids	Systemic anticoagulation via inhibition of vitamin K reduction	Kubo [2005] (72) Noth [2012] (73) Tomassetti [2013] (74)	Kubo: PN (0.5 to 1.0 mg/kg/day for 4 weeks, with subsequent tapering of the dose to 10 to 20 mg/day over a 1-month period) + Warfarin with targed INR between 2.0 to 3.0 Noth and Tomassetti: Warfarin with targed INR between 2.0 to 3.0	Kubo: randomized open label trial prednisolone + warfarin/low molecular weight heparin(n=23) *vs.* prednisolone + placebo (n=33) in stable IPF Noth: ACE-IPF trial: double-blind, randomized, controlled trial of warfarin (n=72) *vs.* placebo (n=73) in stable IPF Tomassetti: retrospective review of stable IPF patients on anticoagulant (n=23) *vs.* not taking anticoagulants (n=79)	Kubo: Mortality from AE-IPF in warfarin *vs.* placebo groups: 18% *vs.* 71% (2 in 11 AE-IPF *vs.* 15 in 21 AE-IPF, P=0.008) Noth: Incidence of AE-IPF in warfarin *vs.* placebo: n=8.3% *vs.* n=2.7%, respectively, P=0.17 Tomassetti: No significant difference in incidence of AE-IPF in anticoagulant *vs.* non-anticoagulant groups: 22% *vs.* 23% (5 of 23 *vs.* 18 of 79 patients) No significant difference in AE-IPF mortality rate: 60% *vs.* 66%, (3 of 5 *vs.* 12 of 18 patients)

Table 2 (*continued*)

Table 2 (*continued*)

Agents for prevention of AE-IPF	Potential mechanisms of action	Selected study or retrospective series	Dose	Study design and number of subjects	Outcome
Azithromycin	Macrolide antibiotic with antiinflammatory qualities, inhibits some Gram-positive bacteria, some Gram-negative bacteria, and many atypical bacteria	Liu [2011] (75)	Azithromycin (oral, dose not available)	Randomized controlled trial of azithromycin (n=50) vs. placebo (n=60) for 12 weeks in stable IPF	After 12 weeks: Decreased TGF-beta1 level in azithromycin group patients AE-IPF admissions: 8% vs. 22% in azithromycin group vs. control group
Nintedanib (formerly BIBF1120)	Angiokinase inhibitor targeting proliferative growth factors in fibroblasts (FGFR, PDGFR, VEGFR)	Richeldi [2014] (16)	Nintedanib 150 mg/day	Two randomized, double-blind, multi-center, placebo-controlled trials in stable IPF: INPULSIS-1 nintedanib group n=309; placebo group n=204 INPULSIS-2 nintedanib group n=329; placebo group n=219 Pooled data nintedanib group n=638; placebo group n=423	Inconsistent results with respect to incidence of and time to first AE-IPF between the 2 trials Time to first AE-IPF INPULSIS-1: no difference between nintedanib vs. placebo group (HR =1.15, P=0.67) INPULSIS-2: longer time to first AE-IPF in nintedanib group (HR 0.38; P=0.005) Frequency of AE-IPF in nintedanib vs. placebo groups INPULSIS-1 nintedanib vs. placebo groups: 3.6% vs. 9.6% INPULSIS-2 nintedanib vs. placebo groups: 6.1% vs. 5.4% Pooled data on time to first AE-IPF No difference in time to first AE-IPF in nintedanib group vs. placebo group (HR=0.64, P=0.08) Smaller proportion of patients with AE-IPF in nintedanib group (4.9% vs. 7.6% in placebo group)

Table 2 (*continued*)

Table 2 (*continued*)

Agents for prevention of AE-IPF	Potential mechanisms of action	Selected study or retrospective series	Dose	Study design and number of subjects	Outcome
Pirfenidone	Inhibition of TGFβ, anti-inflammatory, antioxidant	Azuma [2005] (14) Taniguchi [2010] (76) Noble [2011] (77) King [2014] (15)	Azuma: pirfenidone (titrated from 600 up to 1,800 mg/day) or placebo Taniguchi: pirfenidone (1,200 or 1,800 mg/day) or placebo Noble: Study 004: pirfenidone (1,197 or 2,403 mg/day) or placebo Study 006: pirfenidone (2,403 mg/day) or placebo	Azuma: randomized, double-blind trial of pirfenidone (n=72) vs. placebo (n=35) in stable IPF Taniguchi: randomized, double-blind trial of pirfenidone in high dose (n=110) vs. low-dose (n=56) vs. placebo (n=109) for stable IPF Noble: two randomized, double-blind trials of pirfenidone	Azuma: incidence of AE-IPF significantly lower in pirfenidone group vs. placebo group (0% vs. 14%; P=0.0031). Trial is stopped early for this reason. Taniguchi: no significant difference in the incidence of AE-IPF in high dose vs. low dose vs. placebo groups (5.6% vs. 5.5% vs. 4.8%) Noble: no significant difference in time to worsening IPF (including AE-IPF, death, transplant, or respiratory hospitalization) King: did not assess effect on AE-IPF
N-acetylcysteine	Repletion of glutathione stores to restore natural oxidant/anti-oxidant balance	IPF Net [2014] (17)	NAC 600 mg 3 per day	Prospective, double-blinded, randomized placebo-controlled trial of NAC (n=133) vs. placebo (n=131) in stable IPF over 60 weeks	Incidence of AE-IPF in NAC vs. placebo groups: 2.3% vs. 2.3%, P>0.99
Anti-acid therapy	Prevention of microaspiration due to acid reflux	Lee [2013] (78)	Routine use of anti-acid therapy (PPI or H2B)	Analysis of placebo arms of 3 randomized, controlled trials of various therapies in stable IPF Total patients in placebo arms n=242; total patients taking anti-acids at baseline, before randomization into trial n=124 (PPI, n=113; H2B, n=11)	Incidence of AE-IPF in anti-acid vs. no-anti-acid groups*: 0% vs. 7.6%, P<0.01; Patients with GER diagnosis in anti-acid vs. no-anti-acid groups*: 89% vs. 25%

*94% of patients in antacid group continued this therapy throughout the study and 16% of patients in the no-antacids group started anti-acid therapy during the study. ACE-IPF, anticoagulant effectiveness in idiopathic pulmonary fibrosis; AE-IPF, acute exacerbation of IPF; AZA, azathioprine; FGFR, fibroblast growth factor receptor; GER, gastroesophageal reflux; H2B, histamine-2 blockers; INPULSIS, Safety and Efficacy of BIBF 1120 at High Dose in Idiopathic Pulmonary Fibrosis Patients; IRR, incidence rate ratio; NAC, N-acetylcysteine; PDGFR, platelet derived growth factor receptor; PN, prednisolone; PPI, proton pump inhibitors; TGF-beta, transforming growth factor-beta; VEGFR, vascular endothelial growth factor receptor.

discharge. Four of the five patients in the tacrolimus group and one of the ten patients in the non-tacrolimus group survived their initial or subsequent AE during the review period (57). Median survival in the tacrolimus group was significantly longer than in the non-tacrolimus group (>92 *vs.* 38 days, respectively, P<0.05). In contrast to the fact that no surviving patients from the tacrolimus group had a repeat exacerbation from at least 3 and up to 6 months after their initial AE, four of the non-tacrolimus group patients died from re-exacerbation between 1 to 4.5 months after their initial AE. The authors postulate that tacrolimus may restrain the fibrotic phase of DAD that is characterized by remodeling, thereby averting subsequent exacerbations. Larger, multi-centered randomized studies are needed to fully understand the efficacy of tacrolimus in the treatment of AE-IPF.

Cyclosporine A and corticosteroids

Like tacrolimus, cyclosporine A (CsA) binds to and inhibits calcineurin, restricting lymphocyte proliferation by down regulating transcription of IL-2, IL-3 and IL-4, tumor necrosis factor (TNF)-alpha, CD40 ligand, granulocyte-macrophage colony-stimulating factor, and interferon-gamma (87,88). *In vitro*, however, these effects of CsA are 100 times less powerful than those exhibited by tacrolimus (57). The usefulness of CsA in AE-IPF has been evaluated in a few small non-randomized retrospective studies. Inase *et al.* (58) evaluated thirteen patients with AE-IPF, seven of whom received CsA (1.0-2.0 mg/kg per day) after treatment with corticosteroids (pulse therapy with methylprednisolone 1,000 mg per day for 3 days followed by oral prednisone 40-60 mg per day and maintained for 4 to 8 weeks). They reported a survival of between 60 and 208 weeks in 4 of 7 AE-IPF patients treated with CsA. In contrast, all six patients who did not receive CsA died within sixty-six weeks after the onset of AE-IPF. Homma *et al.* (61) reported that while 34 of 35 AE-IPF patients in a historical comparative cohort had died within 4 months of the exacerbation, 6 of 9 AE-IPF patients treated with CsA (50-200 mg/day combined with corticosteroids for at least 7 days) survived from 7 up to 35 months after the event. A more recent study also reported a better mean survival for AE-IPF patients treated with CsA (low dose 100-150 mg/day) and corticosteroids (pulse therapy with methylprednisolone 1,000 mg per day for 3 days followed by maintenance dosage 0.5-1.0 mg/kg) (285 days, n=11) than those received corticosteroids alone (60 days, n=11) (56).

Large randomized multi-centered studies are still needed to understand the potential role of CsA in the treatment of AE-IPF.

Cyclophosphamide and corticosteroids

Cyclophosphamide is an immunomodulatory alkylating agent used in preventing graft-*vs.*-host disease and thought to spare regulatory T cells (89). Recent cancer research has focused on therapies that ablate the immunocompetent cells found in many tumors that convey immunosuppressive activity in a microenvironment that is consistent with the end of wound healing (90,91). Cyclosphosphamide is one such agent. A few case series involving different definitions of AE-IPF document the use of cyclophosphamide (as single intravenous bolus of 500-750 mg/kg) and corticosteroids (pulsed methylprednisolone from 0.5-1 g/day) in addition to various supportive care measures for AE-IPF (29,30,59). These case series report mixed results, most commonly no significant association between this therapy and significantly improved outcomes. More recently, Morawiec *et al.* (60) retrospectively evaluated the utility of combined pulse methylprednisolone therapy followed by pulse cyclophosphamide therapy in ten AE-IPF patients and seven IPF patients with sub-acute exacerbation (SAE-IPF, with an onset of symptoms as between 30-90 days prior to treatment). Treatment consisted of 1,000 mg methylprednisolone on days 1-3 followed by a cyclophosphamide infusion (500 mg) on day 4, increasing by 200 mg every 2 weeks up to 1,500 mg. The authors reported a 3-month survival of 50% in AE-IPF patients and 100% in SAE-IPF patients, and a 6-month overall survival of 56%, promising some benefit of this combination therapy (60).

Sivelestat and corticosteroids

Neutrophils have been implicated in the pathogenesis of bleomycin-induced pulmonary fibrosis (92), and resistance to bleomycin-induced pulmonary fibrosis has been observed in neutrophil elastase knockout mice (93). The utility of a neutrophil elastase inhibitor, sivelestat, in combination with corticosteroids was examined in a small non-randomized study of ten mechanically ventilated patients with AE-IPF (13). All patients were followed for 180 days and treated with sivelestat and methylprednisolone pulse therapy for 3 days, with subsequent maintenance therapy with prednisone (0.5 mg/kg/day). Four of ten patients survived to day 180. In

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these survivors, the arterial oxygen tension (PaO_2)/inspiratory oxygen fraction (FiO_2) (P/F ratio), peak end expiratory pressure (PEEP) levels, and the values of peripheral white blood cell number and CRP were significantly improved on day 7 as compared to baseline. Larger, multi-centered studies are still clearly needed to determine whether sivelestat is of clinical and survival value in AE-IPF.

Azathioprine and corticosteroids

Azathioprine, a widely used cytotoxic drug that blocks the function of proliferating cells such as T cells and B cells and also decreases the number of circulating monocytes and granulocytes (94), can be used as a steroid-sparing agent in AE-IPF, although there is a dearth of data to support this. A single case report of the use of azathioprine in the treatment of AE-IPF has been published (62). The patient received pulse therapy with methylprednisolone followed by prednisolone (0.8 mg/kg) and azathioprine (15 mg/kg). The patient had marked improvement in hypoxia and chest X-ray findings after five weeks. Azathioprine use in stable IPF, however, is not associated with prevention of AE-IPF. The PANTHER-IPF trial (NCT00650091) (17,71), demonstrated that patients treated with triple therapy with prednisone, azathioprine, and NAC experienced significantly more AE-IPF events than those on placebo.

Antibiotics

The role of the respiratory microbiome has been only scantily investigated in AE-IPF (45). In studies looking at treatment and outcomes in AE-IPF, the vast majority of people received empiric antibiotics in addition to corticosteroids despite the absence of any controlled study demonstrating a benefit with empiric treatment (27). The use of antibiotics in patients with AE-IPF is largely based on the fact that many patients present with fever, flu-like symptoms, and have elevated neutrophil counts in BAL fluid when bronchoscopy is performed (6,36). Unfortunately, this typically leads to prolonged antibiotic courses in patients with AE-IPF in whom no pathogens have been identified, an approach that has been associated with increased risk for subsequent fungal infections and a higher incidence of drug-resistant organisms (95). Procalcitonin, a peptide more abundantly present in the setting of microbial toxins and bacterial proinflammatory molecules, is useful in detecting whether the cause of inflammation is bacterial

in origin and in guiding the initiation and discontinuation of antibiotics in patients with acute respiratory infections (96,97). A recent study evaluated the utility of procalcitonin values to guide the use of antibiotics in AE-IPF (63). Antibiotic therapy guided by a procalcitonin threshold of 0.25 ng/mL resulted in a reduction of antibiotic treatment duration (8.7±6.6 vs. 14.2±5.2 days, P<0.001) as well as fewer patients being exposed to antibiotic treatment (26 of 33 procalcitonin group patients vs. 35 of 35 standard care group patients, P<0.001), in the absence of significant differences in treatment success or mortality rates. The specific antibiotics used in these trials were unfortunately not reported, although many were described as broad-spectrum. Azithromycin, an antibiotic known for its anti-inflammatory qualities, has been evaluated for the treatment of IPF. It demonstrated a significant reduction in both fibrosis and restrictive lung function pattern in a bleomycin induced pulmonary fibrosis mouse model (98). Although azithromycin use in IPF patients was reportedly associated with a lower rate of AE-IPF admissions in one study (75), no formal clinical trials examining the use azithromycin in patients with AE-IPF have been reported to date. It appears that antibiotics therapy during the treatment of AE-IPF is best guided by clinical findings and procalcitonin level monitoring.

Nintedanib

Nintedanib inhibits three tyrosine kinase receptor families including platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) (12). It has been proposed that these growth factors activate signal transduction cascades that result in the development of fibrosis and therefore pose an intriguing target for therapy in IPF (99-101). In the Phase IIb TOMORROW trial (NCT00514683), treatment with nintedanib was associated with slower decline in lung function, decrease in the frequency of AE-IPF and improvement in quality of life (12). In one of two subsequent randomized placebo-controlled Phase III clinical trials performed simultaneously (INPULSIS-1 and -2, NCT01335464, NCT01335477), significantly fewer patients treated with a target dose of 150 mg twice daily of nintedanib experienced an AE-IPF event as compared to those receiving placebo (3.6% vs. 9.6%, respectively), and patients treated with nintedanib had a significant delay in time to first exacerbation (hazard ratio, 0.38; 95% CI, 0.19 to 0.77; P=0.005). When data from the two trials

were pooled, nintedanib treatment was associated with a significantly longer time to adjudicated AE. However, because these effects were not observed in the other study, it is unclear whether nintedanib may serve to prevent exacerbations or significantly delay the time to first exacerbation. Nintedanib has not been formally investigated as a therapy for AE-IPF.

Anticoagulants

Inflammation and vascular injury, including the loss of epithelial cell integrity, have been demonstrated in fibrotic lung disease and therefore the presence of thrombosis in the pulmonary vasculature may also be present (72). In addition, the coagulation cascade is thought to marshal subsequent inflammatory and fibroproliferative responses during normal wound healing (102). The coagulation, inflammation, fibroproliferation and tissue remodeling associated with normal wound healing response may, as a result of repeated tissue injury or aberrant repair mechanism in IPF lungs, result in excessive deposition of extracellular matrix proteins (102). Treatment of IPF with anti-coagulants has produced mixed results. An initial study examined the effects of treatment with corticosteroids alone or in combination with anticoagulant therapy (72). The authors reported better survival in those receiving anticoagulation, due in large part to improved survival in subjects with AE-IPF. The mortality from AE-IPF in the anticoagulant group, (18%, 2 in 11 AE) was significantly reduced compared to that of the non-anticoagulant group (71%, 15 in 21 AE). This study, however, has been criticized based on low subject retention rate, high incidence of AE overall, and lack of blinding (18,103). A follow up double-blind, randomized, placebo-controlled trial of warfarin, the ACE-IPF trial, showed warfarin to be ineffective and potentially harmful as a therapy for patients with IPF and AE-IPF (73). AE of IPF were noted to occur in a greater number of subjects in the warfarin group compared with the placebo group (n=6 *vs.* n=2, respectively, P=0.17), and there were no significant treatment effects observed in the secondary endpoints (FVC, 6-minute walk distance, and carbon monoxide diffusion). The trial was ended early due to the low probability of benefit and an increase in mortality in the warfarin group. Another retrospective cohort study by Tomassetti *et al.*, demonstrated that patients with IPF treated with anticoagulants had worse survival and shorter interval to disease progression (74).

More recently, increased deposition of total lung collagen in an animal model of viral exacerbation of pulmonary fibrosis in which the extrinsic coagulation cascade was upregulated has been reported in an abstract (104). The authors suggest that, when taken together with the failure of the studies employing systemic anticoagulants described above, coagulation activity may be both harmful and beneficial in pulmonary fibrosis and that anticoagulant interventions should thus be targeted toward specific profibrotic processes.

Thrombomodulin, a protein expressed by epithelial cells, functions as a cofactor that binds to thrombin to greatly increase the activation of protein C. Recombinant human thrombomodulin (rhTM) is approved as a treatment for disseminated vascular coagulopathy in Japan. After confirming that AE-IPF patients in a historical cohort exhibited signs of hypercoagulability, Tsushima *et al.* (64) prospectively evaluated the utility of rhTM in 20 patients with AE-IPF. Six historical cases of AE-IPF patients not treated with rhTM were used for comparison. All patients received methylprednisolone pulse therapy (1 g/day for three days) followed by prednisolone (1 mg/kg/day), and were on positive pressure ventilation. The patients in the treatment group also received 0.06 mg/kg/day rhTM for 6 days). The 28-day mortality was significantly higher in untreated patients as compared to those receiving rhTM (83% *vs.* 35%, P=0.048). The authors also reported improved SpO_2/FiO_2 and a reduction in the degree of intravascular coagulation disturbance in association to rhTM administration (64). These results are encouraging and merit further exploration to define the role of thrombomodulin in the treatment of AE-IPF.

Pirfenidone

Pirfenidone has demonstrated anti-oxidant, anti-inflammatory, and antifibrotic effects in experimental models of pulmonary fibrosis (14). An initial prospective trial involving a group of 107 IPF patients was completed in Japan (14). Patients received either pirfenidone or placebo in a dose-titration schedule (from 600 up to 1,800 mg/day). While the study did not reach its primary endpoint of improvement in the lowest oxygen saturation during 6-minute exercise testing, a positive treatment effect in the secondary endpoints of change in vital capacity at 9 months and a lower incidence of AE-IPF events in the treatment group was observed, with all five episodes of AE-IPF at the 6 months interim analysis occurring in the placebo group. This study was discontinued in favor of pirfenidone based largely on the notion that

it could prevent AE-IPF. Unfortunately, larger follow-up trials (Taniguchi *et al.*, and the CAPACITY-1 and -2 studies NCT00287729 and NCT00287716) did not confirm the reduction in incidence of AE-IPF (dosing of pirfenidone in the latter trials ranged from 1,197 to 2,403 mg/day) (76,77). Another subsequent phase 3 trial, the ASCEND study (NCT01366209), documented improvement in stable IPF, but did not assess effective treatment of AE-IPF (15). Currently there are no compelling data to support the use of pirfenidone in AE-IPF.

Hemoperfusion with polymyxin B-Immobilized fiber column

One potential therapy for AE-IPF currently under study is direct hemoperfusion with a polymyxin B (PMX)-immobilized fiber column (PMX-DHP). PMX-DHP columns absorb endotoxins and reactive oxygen species (ROS), amongst other substances, and also selectively remove activated neutrophils, reducing the ability of circulating cells to cause endothelial damage (105-107). The use of PMX-DHP columns has been studied in patients with ARDS, which, like AE-IPF, is characterized by DAD, and improvement in oxygenation has been observed after therapy (108,109). The use of PMX-DHP for AE-IPF has been explored in Japan. An open-label pilot study and a case report including a total of seven AE-IPF patients indicate that therapy is safe and might be of benefit (65,66). In addition, a retrospective review of 19 AE-IPF subjects reported a median survival of 22 days after diagnosis of AE in polymyxin-treated patients. Survival rates after diagnosis of AE were 47%, 32% and 26% at 1, 2, and 3 months, respectively. Serum levels of IL-7, an inhibitor of fibroblast TGF-β production and signaling were significantly increased in the surviving subjects, possibly indicating an anti-fibrotic mechanism in the action of PMX-DHP (67,110). A larger retrospective study of 160 patients with interstitial pneumonia (IP), that included 73 patients with IPF demonstrated that, in patients with AE-IPF, P/F ratio was significantly improved after treatment with PMX-DHP compared to pre-PMX-DHP (173.9±105.4 to 195.2±106.8 Torr, respectively, P=0.003) (68). A subsequent study examined the possible mechanism of action of improvement in oxygenation by PMX-DHP by examining the cytokine profile adsorbed onto the PMX-DHP fibers (69). They found a significant reduction in serum levels of cytokines including IL-9, IL-12, IL-17, PDGF and VEGF, with IL-12 and VEGF the most significantly reduced. The authors postulate that the observed therapeutic effects of PMH-DHP are based

on the adsorption of proinflammatory, profibrotic, and proangiogenic cytokines by PMX-DHP-fibers. Specifically, the removal of VEGF may contribute to the improvement in oxygenation by suppressing vascular permeability in the lung. Finally, a recent abstract reported that the 3-month survival of patients treated with PMX-DHP was better than those not receiving this treatment (72% of 14 patients *vs.* 48% of 18 patients, respectively, HR 0.33, P=0.04) (111). Although direct hemoperfusion with PMX-DHP therapy is promising, larger, randomized multicenter trials are still needed to determine its role in AE-IPF.

Plasma exchange, rituximab, and corticosteroids

The pathogenesis of AE-IPF has also been linked to antibody-driven autoimmunity leading to epithelial cell apoptosis (112). A variety of unconventional IgG autoantibodies have been observed in as many as 80% of IPF patients, and some, like anti-heat shock protein 70, are associated with increased mortality and pulmonary function deterioration (113,114). Reducing the presence of antibodies or deposition of immune complex could therefore be of benefit in AE-IPF. Two studies to test the feasibility of autoantibody removal by plasma exchange (PEX) together with immunoglobulin load reduction by B-cell depletion with rituximab, in combination with or without anti-inflammatory corticosteroids for the treatment of AE-IPF are currently being conducted (NCT01266317 and NCT01524068). Recently, the outcomes of six AE-IPF subjects treated with PEX and rituximab were published in abstract form. All received intravenous methylprednisolone (1 g), then an oral taper from 40 mg/day over 4 weeks and 5 PEX (1.5 times plasma volume) over six days, followed by rituximab (1 g intravenously, repeated after 1 week) (70). Results of these studies will help to guide larger, multicenter trials to ascertain the therapeutic utility of autoantibody reduction in AE-IPF.

Anti-acids

Microaspiration of gastroesophageal refluxate could be one of the key insults to the delicate lung parenchyma that leads to IPF and/or the cause of AE-IPF. The prevalence of GER in IPF patients is high, and pepsin has been found in the bronchoalveolar lavage fluid of patients with AE-IPF (37,115). An analysis of the placebo arms of three prospective, randomized, controlled trials of various therapeutic agents in stable IPF found that patients taking

antacids routinely (proton pump inhibitors or histamine-2 blockers at baseline, prior to randomization into a trial), were less likely to develop AE-IPF than patients not taking anti-acids (0 of 124 patients on anti-acids *vs.* 9 of 118 patients not on anti-acids) (78). Although further prospective study is warranted, this analysis suggests that control of gastroesophageal reflux with anti-acid therapy could be useful in preventing AE-IPF.

N-acetylcysteine (NAC)

NAC, an antioxidant, has been studied as a potentially therapeutic agent in IPF with the expectation that it could prevent the oxidative injury that precedes fibroproliferation by restoring the natural oxidant/antioxidant balance. In the PANTHER-IPF trial (17) (NCT00650091), oral NAC therapy (1,800 mg/day) did not result in a reduction in the incidence of AE-IPF. Patients talking NAC were in fact significantly more likely to develop a cardiac adverse event as compared to patients on placebo (6.8 % in the NAC group *vs.* 1.5% in the placebo group, P=0.03). NAC has not been considered as a therapy for use during an AE-IPF.

Non-invasive ventilation

Previous studies have suggested that there is no benefit to MV in patients with IPF presenting with acute respiratory failure and should be restricted to those patients who can have lung transplantation within several days of initiating MV (116). Given the high mortality rate associated with invasive MV, a small study involving 11 patients was performed to describe the outcomes in patient with AE-IPF who received noninvasive ventilation (NIV) (117). Five patients were able to avoid intubation and survived more than 3 months after their AE. Of the six patients who failed NIV, four required intubation and all died within 3 months. This suggests there may be a role for NIV in AE-IPF but further study is clearly needed.

Intraoperative management for AE-IPF prevention

Postoperative exacerbation of IPF (PAE-IPF) is a recognized complication after pulmonary resection and carries a high mortality rate (43,118,119). Appropriate intraoperative management of these patients is therefore profoundly important to prevent PAE-IPF and improve mortality. One area of interest is intraoperative fluid balance. Mizuno *et al.* retrospectively analyzed 52 patients

with clinical IPF who underwent pulmonary resection for primary lung cancer (120). The incidence of PAE-IPF was 13.5% (7 of 52 patients), with six of the seven patients dying of respiratory failure. The authors reported an increased amount of intraoperative fluid infused (7.71±3.11 *vs.* 10.0±3.66 mL/kg/h, P=0.049) and intraoperative fluid balance (4.99±2.86 *vs.* 8.00±4.21 mL/kg/h) in the patients who developed PAE-IPF. A multivariate logistic analysis of all patients showed that increased intraoperative fluid balance was a prognostic factor for PAE-IPF (OR 1.312, P=0.026).

The influence of intraoperative oxygen delivered in patients with IPF has also been discussed as a potential risk factor in development of PAE-IPF. It has been suggested that AE-IPF is similar to acute lung injury/ARDS in that pulmonary injury is closely related to ROS (121). More specifically, high concentrations of oxygen stimulate the release of inflammatory cytokines including TNF-α, IL-8, IFN-gamma and IL-6 which can injure pulmonary endothelium and alveoli and can lead to pulmonary hemorrhage, lung edema, hyalinization and increased alveolar thickness (121). Ventilator-induced lung injury may also result from barotrauma or volutrauma, resulting in increased recruitment of circulating fibrocytes to the lung and worsening fibrosis (44). High pressure ventilation and inhalation of high oxygen concentrations should therefore be considered with caution in patients with IPF undergoing surgical procedures.

Conclusions

The AE-IPF is a severe and life threatening event that carries a high mortality rate and results in significantly reduced median survival. Current mainstays of AE-IPF treatment are limited and involve supportive care with the addition of corticosteroids, broad spectrum antibiotics and sometimes additional immunosuppression, even though little data exists to support this approach. Novel therapies targeted at inflammatory and fibrogenic mediators, autoimmunity, and fibroblast proliferation have shown promise in decreasing the incidence of AE-IPF or improving mortality from AEs. The combined use of tacrolimus and corticosteroids, removal of select immune system cells and mediators with PMX-DHP, and procalcitonin-guided antibiotic use are amongst the most promising. While these studies are encouraging, it is unfortunately not clear at this time whether treatment with any of the therapies discussed in this review will ever prove efficacious for AE-IPF under

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the gold standard of a prospective, randomized, controlled clinical trial. This is mainly due to the great challenge that conducting such a trial would represent in this rare disorder that lacks a simple or uniform diagnostic method. Nevertheless, there is evidence to suggest that the prompt initiation of oxygen therapy, corticosteroids, antibiotics, and/or cyclophosphamide, is associated with a better prognosis (122). Given the likely complex pathophysiology of AE-IPF, it is also possible that a therapeutic approach involving multiple therapeutic modalities will result in better treatment outcomes. Advances in the development of new therapeutic agents for stable IPF are hoped to translate into a better understanding of the pathogenic mechanisms of AE-IPF, and subsequently to result in improved therapeutics and prognosis for this devastating condition.

Acknowledgements

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Adverse effects of outdoor pollution in the elderly

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Abstract: With fewer newborns and people living longer, older people are making up an increasing fraction of the total population. Epidemiological evidence shows that older-age-related health problems affect a wide and expanding proportion of the world population. One of the major epidemiological trends of this century is the rise of chronic diseases that affect more elderly than younger people. A total of 3.7 million premature deaths worldwide in 2012 are attributable to outdoor air pollution; the susceptibility to adverse effects of air pollution is expected to differ widely between people and within the same person, and also over time. Frailty history, a measure of multi-system decline, modifies cumulative associations between air pollution and lung function. Moreover, pre-existing diseases may determine susceptibility. In the elderly, due to comorbidity, exposure to air pollutants may even be fatal. Rapid and not-well-planned urbanization is associated with high level of ambient air pollution, mainly caused by vehicular exhausts. In general, there is sufficient evidence of the adverse effects related to short-term exposure, while fewer studies have addressed the longer-term health effects. Increased pollution exposures have been associated with increased mortality, hospital admissions/ emergency-room visits, mainly due to exacerbations of chronic diseases or to respiratory tract infections (e.g., pneumonia). These effects may also be modulated by ambient temperature and many studies show that the elderly are mostly vulnerable to heat waves. The association between heat and mortality in the elderly is well-documented, while less is known regarding the associations with hospital admissions. Chronic exposure to elevated levels of air pollution has been related to the incidence of chronic obstructive pulmonary disease (COPD), chronic bronchitis (CB), asthma, and emphysema. There is also growing evidence suggesting adverse effects on lung function related to long-term exposure to ambient air pollution. Few studies have assessed long-term mortality in the elderly. It is still unclear what are the pollutants most damaging to the health of the elderly. It seems that elderly subjects are more vulnerable to particulate matter (PM) than to other pollutants, with particular effect on daily cardio-respiratory mortality and acute hospital admissions. Not many studies have targeted elderly people specifically, as well as specific respiratory morbidity. Most data have shown higher risks in the elderly compared to the rest of the population. Future epidemiological cohort studies need to keep investigating the health effects of air pollutants (mainly cardiopulmonary diseases) on the elderly.

Keywords: Outdoor air pollution; elderly people; frailty elderly; respiratory disease; environmental exposure

Introduction

With fewer newborns and people living longer, older people are making up an increasing share of the total population. Population aging is a transforming force, and we must prepare for a new demographic reality.

Globally, the proportion of older persons aged 80 years or over (the oldest old) within the elderly population was 14% in 2013 and is projected to reach 19% in 2050. If this projection holds true, there will be 392 million persons aged

80 years or over by 2050, more than three times the present (1).

Epidemiological studies show that health problems associated with aged populations affect a wide and expanding proportion of the world population; one of the major epidemiological trends of this century is the rise of chronic diseases (2).

Given these demographic perspectives, effective prevention is necessary to keep the elderly population in good health for as long as possible. We need to improve our understanding of the health consequences associated with the exposure to various risk factors, especially in vulnerable persons as the elderly.

Ageing is a continuous process of progressive decline of the body's function leading to increased vulnerability, frailty or sensitivity of elderly people. Moreover, with increasing age, the immune system undergoes alterations culminating in a progressive deterioration in the ability to respond to infection and vaccination, with consequent increases in morbidity and mortality due to infectious diseases among elderly persons (3).

Outdoor air pollution and health effects

Outdoor air pollution is a major environmental health problem affecting people with 3.7 million premature deaths worldwide in 2012. About 88% of premature deaths occurred in low- and middle-income countries, with the largest number in the World Health Organization (WHO) Western Pacific and South-East Asia regions (4). WHO estimates that premature deaths related to air pollution are due for about 80% to ischaemic heart disease and strokes, for 14% to chronic obstructive pulmonary disease (COPD) or acute lower respiratory infections, and for 6% to lung cancer.

A 2013 assessment by the WHO's International Agency for Research on Cancer (IARC) concluded that outdoor air pollution is carcinogenic to humans, with particulate matter (PM) closely associated with increased cancer incidence, especially cancer of the lung (4).

Susceptibility to adverse effects of air pollution is expected to differ widely between people and within the same person, over time. While some individuals may experience no symptom or only clinically irrelevant changes, a similar exposure may trigger serious exacerbations of health problems among the frail subjects. Reduced lung function occurs as a natural part of aging and there is scientific evidence that elderly people are largely affected by the increased impairment resulting from exposure to air

pollutants (5). Frailty history, a measure of multi-system decline, modifies the cumulative associations between air pollution and lung function (6).

Moreover, pre-existing diseases may determine susceptibility. Elderly people will most likely suffer from chronic diseases, and there is evidence that co-existing chronic lung, heart or circulatory conditions may worsen following exposure to environmental pollutants (7,8).

It is still unclear what pollutants are the most damaging to the health of the elderly. Elderly subjects in the EpiAir study (Italian epidemiological surveillance on ambient pollution and health) were found to be more vulnerable to PM_{10} than to other pollutants (9). Another study observed that $PM_{2.5}$ was 3-fold more noxious than PM_{10}, suggesting that fine particulates may constitute a major public health issue in the elderly, even in concentrations lower than the current limit values (10). A study conducted in Finland in 65 years old and above subjects has evaluated which particle size fractions and sources of particles are responsible for the health effects, particularly for daily cardio-respiratory mortality and acute hospital admissions. It reported that all particle fractions can contribute to adverse respiratory health effects among the elderly. Overall, associations were stronger for respiratory than for cardiovascular outcomes. In particular, most particle fractions had positive associations with admissions for pneumonia and asthma/COPD. The strongest associations were found for particles with diameter 0.1-0.29 μm, with 3.1% increase [95% confidence interval (CI), 0.43-5.8] for pneumonia over the 5-day mean, and 3.8% (95% CI, 1.3-6.3) for asthma-COPD at lag 0, for an inter-quartile increase in particles (11).

The second phase of the Air Pollution on Health: a European Approach (APHEA 2 project, 1990s) analyzed hospital admissions for respiratory diseases in eight European cities. Daily ozone (O_3) levels accounted for a large proportion of the between-cities variability of the PM_{10} effect estimates in elderly people (≥65 years). The authors have suggested that secondary particles, formed by the same photochemistry that produces O_3, might be responsible for the observed particle effects in the elderly (12).

Short-term effects

The association between short-term exposure to air pollution and acute health effects has been mainly investigated through time-series studies by evaluating the effects on a population of defined size over a period of exposure assessment (13).

Short-term morbidity

There is strong evidence of an association between short-term exposure to air pollutants and respiratory morbidity in the elderly (14). Symptoms may appear several days following an increased exposure level and may persist for a number of days.

Below we highlight the findings of some of these studies.

In the second half of the 1980s, a study performed in Barcelona (Spain) evaluated the relationship between daily emergency-room admissions for COPD for the period 1985-1989 (70% were over age 65) and ambient sulphur dioxide (SO_2). It was found that an increment of 25 µg/m³ in 24-hour average SO_2 concentration produced an increase of 6% and 9% in admissions during winter and summer, respectively. For black smoke (BS), a similar change was found during winter, whereas the change was smaller in summer (15).

Subsequently, other studies have been performed to assess short-term effects of outdoor air pollution on morbidity in elderly people (*Table 1*).

Schwartz *et al.* showed significant positive associations between respiratory hospital admissions and levels of SO_2, PM_{10} and O_3 in persons aged 65 years or older living in New Haven and Takoma (U.S., 1988-1990). Among the pollutants measured, the association was strongest with PM_{10}, followed by O_3 (16).

Medina-Ramón *et al.*, in a very large case cross-over study of elderly people (≥65 years), carried out in 36 U.S. cities [1986-1999], found that short-term increases in O_3 and PM_{10} ambient concentrations were related to increased hospital admissions for COPD and pneumonia, especially during the warm season (17).

In elderly people with permanent residence in Boston (U.S.) during the period 1995-1999, Zanobetti *et al.* reported the risk of hospital admission for pneumonia to be positively and significantly associated with background black carbon (BC), $PM_{2.5}$, and carbon monoxide (CO), but only in the winter (18).

In the 1990s, studies on elderly people were also performed in Vancouver (Canada), a city with relatively low levels of PM_{10} (*Table 1*).

The study of Yang *et al.* [1994-1998] showed that nitrogen dioxide (NO_2), CO, and PM_{10} were significantly linked to acute hospitalizations for COPD. The magnitude of effects increased slightly with increasing days of exposure. This study did not find significant association between either SO_2 or O_3 and COPD hospitalizations (19).

Still in Vancouver, Chen *et al.* found that, in the period

1995-1999, in addition to PM_{10}, $PM_{2.5}$ was significantly associated with COPD hospitalizations in elderly people (20).

Villeneuve *et al.*, in a study conducted in Edmonton (Canada, 1999-2002), estimated that 8.1% of 57,912 emergency department (ED) visits for asthma occurred among elderly people. In this population, patients aged 75 years and older, an increase in the 5-day average level of NO_2 was associated with a significant increase of asthma-related ED-visits. The same occurred for the same-day average level of $PM_{2.5}$ (21).

In the period 1999-2002, a large study was conducted to examine the link between fine particle air pollution and hospital admissions for heart- and lung-related illnesses. The study analyzed hospital admissions of all elderly residents (>65 years) in 204 U.S. counties. Short-term exposure to $PM_{2.5}$ (from such sources as motor vehicle exhaust and power plant emissions) significantly increased the risk for cardiovascular and respiratory diseases. Day-to-day variation in $PM_{2.5}$ concentration was associated with changes in number of hospital admissions for cardio-respiratory outcomes, for at least 1-day exposure lag. For respiratory outcomes, the largest effects occurred at lags 0-1 days for COPD (median day hospitalization rate per 100,000: 2.6) and at lag 2-day for respiratory tract infections (rate: 5.4). Participants aged 75 years or older experienced larger increases in admissions for heart problems and COPD. Importantly, this study showed that even small increases in $PM_{2.5}$ (just based on natural day-to-day variations) resulted in increased hospital admissions (25).

Furthermore, studies conducted in major hospitals of Hong Kong have shown associations between the risk of emergency hospital admissions for respiratory diseases and outdoor pollution. In 1994-1995, admissions for all respiratory diseases and COPD were related to levels of SO_2, NO_2, O_3, and PM_{10}, whereas admissions for asthma, pneumonia, and influenza were positively and significantly associated with NO_2, O_3, and PM_{10}; people aged ≥65 years were at higher risk (26). Data collected from 2000 to 2004 indicated that a 10 µg/m³ increase in PM_{10} and $PM_{2.5}$ was associated, respectively, with 2.4% and 3.1% increase in hospital admissions for acute exacerbation of COPD at cumulative lag days of 0-5; O_3 contributed to the largest risk for admission [relative risk (RR), 1.034; 95% CI, 1.030-1.040]. A less delayed effect was observed for NO_2 (best lag: cumulative lag days 0-3). The strongest effect of SO_2 was observed at no-lag days (27). Still in Hong Kong, in the period 2000-2005, it was shown that, after exposure to air pollution, the individuals aged 65 years or older, compared

Table 1 Associations between outdoor pollution exposure and morbidity in elderly

Author (study period, country) (references)	Age (years)	Outcome	Pollutant	Increment/threshold	Association	95% CI
Schwartz 1995 (1988-1990, U.S.) New Haven (16)	≥65	Respiratory-H	PM_{10}	50 μg/m³	RR 1.06	1.00-1.13
			SO_2	50 μg/m³	RR 1.03	1.02-1.05
			O_3	50 μg/m³	RR 1.06	0.99-1.13
Schwartz 1995 (1988-1990, U.S.) Tacoma (16)	≥65	Respiratory-H	PM_{10}	50 μg/m³	RR 1.10	1.03-1.17
			SO_2	50 μg/m³	RR 1.06	1.01-1.12
			O_3	50 μg/m³	RR 1.21	1.06-1.38
Medina-Ramón 2006 (1986-1999, U.S.) (17)	≥65	COPD-H	PM_{10}	10 μg/m³	1.47% change	0.93-2.01
		Pneumonia-H		10 μg/m³	0.84% change	0.50-1.19
Zanobetti 2006 (1995-1999, U.S.) (18)	≥65	Pneumonia-H	NO_2	16.8 ppb	12.7% change	5.80-18.40
			$PM_{2.5}$	16.32 μg/m³	8.65% change	1.20-15.38
			BC	1.7 μg/m³	8.34% change	0.20-15.80
Yang 2005 (1994-1998, Canada) (19)	≥65	COPD-H	NO_2	7-day average	RR 1.11	1.04-1.20
			CO	7-day average	RR 1.08	1.02-1.13
			PM_{10}	7-day average	RR 1.13	1.05-1.21
Chen 2004 (1995-1999, Canada) (20)	≥65	COPD-H	$PM_{2.5}$	3-day average	RR 1.09	1.03-1.16
Villeneuve 2007 (1999-2002, Canada) (21)	≥75	ED-visit for asthma	NO_2	IQR—5-days average	OR 1.20	1.04-1.38
			$PM_{2.5}$	IQR—same day average	OR 1.12	1.01-1.23
Larrieu 2009 (2000-2009, France) (22)	≥65	Home visits for URD	PM_{10}	10 μg/m³	ERR 8.3%	2.00-14.70
			NO_2	10 μg/m³	ERR 12.3%	4.90-19.70
Schikowski 2005 (1985-1994, Germany) Women (23)		Cough	Major road	<100 m	OR 1.24	1.03-1.49
		COPD	Major road	<100 m	OR 1.79	1.06-3.02
	55	COPD	PM_{10}	7 μg/m³ in 5-year mean	OR 1.33	1.03-1.72
		COPD	NO_2	7 μg/m³ in 5-year mean	OR 1.43	1.23-1.66
		CB	NO_2	7 μg/m³ in 5-year mean	OR 1.37	1.16-1.62
Bentayeb 2010 (1999-2001, France) (24)	≥65	Cough	PM_{10}	Third quartile	OR 1.33	1.00-1.77
		Cough	SO_2	Third quartile	OR 1.55	1.16-2.08
		Phlegm	SO_2	Third quartile	OR 1.45	1.04-2.01

CI, confidence interval; H, hospitalization; PM, particulate matter; SO_2, sulphur dioxide; O_3, ozone; RR, relative risk; COPD, chronic obstructive pulmonary disease; NO_2, nitrogen dioxide; BC, black carbon; CO, carbon monoxide; ED, emergency department; IQR, inter-quartile range; OR, odds ratio; URD, upper respiratory diseases; ERR, excess relative risk; CB, chronic bronchitis.

to those aged <65 years, had a shorter best lag time to develop asthma exacerbations (28).

Larrieu *et al.* explored the associations between daily levels of NO_2, O_3, and PM_{10} and medical home visits in Bordeaux (France, 2000-2006). Visits for upper- or lower-respiratory diseases represented approximately 20% of the activity of the general practitioners. During the 3 days following a 10 μg/m³ increment in PM_{10}, the excess relative risk (ERR) of home visits increased significantly by 1.5% (95% CI, 0.3-2.7) for upper respiratory diseases, and by 2.5% (95% CI, 0.5-4.4) for lower respiratory diseases. An increased risk of visits for

lower respiratory diseases was observed also for a 10 μg/m³ increment in NO_2 (ERR 2.6%; 95% CI, 0.2-4.9). The risk of visits for respiratory diseases was much higher in the elderly than in other age subgroups. The difference was particularly evident for upper respiratory diseases (*Table 1*) (22).

Concerning the impact on pulmonary function, in a cohort study of 1,100 elderly men [1995-2005] from the U.S. Normative Aging Study (NAS), a 15 ppb increase in ambient O_3 (in the last 8 hours) was associated with a 1.25% decrease in forced expiratory volume in the first second (FEV_1) (95% CI, −1.96-−0.54%) (29).

In summary, the relationship between short-term exposure to air pollution and morbidity in the elderly is well-documented. Significant increases in hospitalizations, ED or home medical visits for respiratory causes, mainly COPD, asthma, and pneumonia, resulted associated with exposures to outdoor air pollutants.

Short-term mortality

Time-series studies based on background air pollution have reported significant short-term effects of air pollution on mortality, as well.

In early 1990-1991, Saldiva and colleagues evaluated the relationship between daily mortality of elderly people ($\geq$65 years) and air pollution in the metropolitan area of Sao Paulo (Brazil). Mortality was associated with PM_{10}, nitrogen oxides (NO_x), SO_2, and CO. The strongest association was with PM_{10}: a 100 $\mu g/m^3$ increase was associated with an increase in overall mortality by approximately 13%. The dose-response relationship between mortality and PM_{10} was almost linear, with no evidence of a "safe" threshold level (30).

Rapid and not-well-planned urbanization is associated with high level of ambient air pollution, mainly caused by increasing emissions of motor vehicles. Latin America is the most highly urbanized region in the developing world. In 2000, about 75% of the population was living in urban areas. The Estudio de Salud y Contaminación del Aire en Latinoamérica (ESCALA project, 2006-2009) assessed the association between exposure to outdoor air pollution and mortality in nine Latin American cities. Meta-analyses showed that PM_{10} was associated with increased mortality in most cities. Larger effects were observed in older people ($\geq$65 years) and for respiratory causes, compared to cardiovascular causes. In the elderly, the increment in daily concentration of PM_{10} augmented the risks for respiratory mortality and for COPD mortality; O_3 was significantly related to increased risk for cardiopulmonary mortality (*Table 2*). The percentage of people $\geq$65 years in that population was one of the variables that best explained the heterogeneity in mortality risks between cities (31). In seven Chilean urban centers during the period 1997-2007, among the older elderly ($\geq$85 years), inter-quartile increases in PM_{10}, $PM_{2.5}$, SO_2, NO_2, CO and elemental and organic carbon were associated with a 2-7% increase in daily mortality. The estimates were even higher (11-19%) among the older elderly who did not complete primary school (40).

Aga *et al.*, by using data collected during the APHEA 2 project (started in 1998), have investigated the effects of PM_{10} and BS on mortality in the elderly from 29 European cities ($\geq$65 years of age). Increments in PM_{10} and BS produced an increase in the daily number of deaths (*Table 2*). The effect magnitude was positively related to the long-term average levels of NO_2, temperature, and to the proportion of the elderly in each city (32).

In the WHO-Database on outdoor pollution, air quality is represented by annual mean concentration of PM_{10} and $PM_{2.5}$. The database covers the period from 2003 to 2010 and includes outdoor air pollution monitoring from almost 1,100 cities in 91 countries. The world's average PM_{10} levels by region range from 21 to 142 $\mu g/m^3$, with a world's average of 71 $\mu g/m^3$.

Most of the cities from India, China, Pakistan, Mongolia and Indonesia are ranked as heavily air-polluted cities (annual mean PM_{10} >100 $\mu g/m^3$) (41).

The relationship between outdoor air pollution and daily mortality in China has been largely examined in the previous century when the predominant source of air pollution was coal combustion (42). China's economy has developed rapidly in the last 20 years, with consequent increases in energy use and industrial wastes. The economic growth has been accompanied by worsening air quality. Total suspended particulates (TSP) and SO_2 have been decreasing in the last decade in several large cities, due to the adoption of various control measures. However, ambient air NO_x level has been increasing due to the increased number of motor vehicles. Ambient air pollution in large cities has changed from the conventional coal combustion type to the mixed coal combustion/motor vehicle emission type (43). Given the changes, studies have been performed more recently in China to investigate the acute effects of outdoor air pollution on mortality outcomes.

As part of the Public Health and Air Pollution in Asia (PAPA) program, a time-series study was conducted in Shanghai (China) to investigate the relationship between outdoor air pollution and daily mortality using 4 years of daily data [2001-2004]. The study concluded that short-term exposure to outdoor PM_{10}, SO_2, NO_2, and O_3 was associated with daily mortality from all natural causes and from cardio-pulmonary diseases. Moreover, the results have provided preliminary evidence that women, older people, and people with a low level of education are more vulnerable to air pollution than men, younger people, and people with a high level of education, respectively (44).

Similar results have been observed in another Chinese city (Wuhan, 4.5 million residents). In the period 2000-2004 there was a consistent association between PM_{10} and mortality, especially for respiratory diseases. In general, the

Table 2 Associations between outdoor pollution exposure and mortality in elderly

Author (study period, country) (references)	Age (years)	Mortality for	Pollutant	Increment	Association	95% CI
Romieu 2012 (2006-2009, Latin America) (31)	≥65	Respiratory causes	PM_{10}	10 µg/m³	RPC 0.72%	0.54-0.89
		COPD	PM_{10}	10 µg/m³	RPC 1.98%	0.78-3.23
		Cardiopulmonary causes	O_3	10 µg/m³	RPC 0.33%	0.20-0.46
Aga 2003 [1998] (32)	≥65	All causes	PM_{10}	10 µg/m³	0.80% change	0.70-0.90
			BS	10 µg/m³	0.60% change	0.50-0.80
Chen (2001-2008, China) (33)	≥65	All causes	PM_{10}	10 µg/m³	0.50% change	0.22-0.78
Fischer 2003 (1986-1994, the Netherlands) (34)	65-74	COPD	BS	40 µg/m³	RR 1.20	1.10-1.32
			SO_2	40 µg/m³	RR 1.14	1.03-1.25
			NO_2	30 µg/m³	RR 1.17	1.10-1.27
			CO	1,200 µg/m³	RR 1.38	1.15-1.65
Fischer 2003 (1986-1994, the Netherlands) (34)	≥75	Pneumonia	PM_{10}	80 µg/m³	RR 1.12	1.01-1.25
			BS	40 µg/m³	RR 1.12	1.05-1.20
			SO_2	40 µg/m³	RR 1.10	1.03-1.17
			NO_2	30 µg/m³	RR 1.13	1.07-1.19
			CO	1,200 µg/m³	RR 1.23	1.09-1.39
			O_3	150 µg/m³	RR 1.37	1.24-1.50
Filleul 2004 (1988-1997, France) (35)	≥65	Respiratory causes	BS	10 µg/m³	9.20% change	3.40-15.30
Naess 2007 (1992-1998, Norway) Men (36)	51-70	COPD	NO_2	Quartile	HR 1.21	1.05-1.39
			PM_{10}		HR 1.29	1.12-1.48
			$PM_{2.5}$		HR 1.27	1.11-1.47
Naess 2007 (1992-1998, Norway) Men (36)	71-90	COPD	NO_2	Quartile	HR 1.04	0.95-1.14
			PM_{10}		HR 1.08	0.98-1.18
			$PM_{2.5}$		HR 1.10	1.00-1.21
Naess 2007 (1992-1998, Norway) Women (36)	51-70	COPD	NO_2	Quartile	HR 1.06	0.92-1.21
			PM_{10}		HR 1.06	0.92-1.22
			$PM_{2.5}$		HR 1.09	0.94-1.25
Naess 2007 (1992-1998, Norway) Women (36)	71-90	COPD	NO_2	Quartile	HR 1.07	0.97-1.17
			PM_{10}		HR 1.08	0.98-1.19
			$PM_{2.5}$		HR 1.05	0.96-1.16
Franklin 2007 (1997-2002, U.S.) (10)	≥75	Respiratory causes	$PM_{2.5}$	10 µg/m³	1.85% change	0.27-3.44
	<75				1.53% change	−0.67-3.74
Brunekreef 2009 (1987-1996, The Netherlands) (37)	55-69	Respiratory causes	NO_2	30 µg/m³	RR 1.37	1.00-1.87
			BS	10 µg/m³	RR 1.22	0.99-1.50
			$PM_{2.5}$	10 µg/m³	RR 1.07	0.75-1.52
Jerrett 2009 (1982-2000, U.S.) (38)	>60	Respiratory causes	O_3	10 µg/m³	RR 1.02	1.00-1.05
Dong 2012 (1998-2009, China) (39)	>60	Respiratory causes	NO_2	10 µg/m³	HR 2.88	2.60-3.20
			$PM_{2.5}$		HR 1.66	1.59-1.74
			SO_2		HR 1.04	0.97-1.13

CI, confidence interval; COPD, chronic obstructive pulmonary disease; PM, particulate matter; O_3, ozone; RPC, risk percent change; BS, black smoke; SO_2, sulphur dioxide; NO_2, nitrogen dioxide; CO, carbon monoxide; RR, relative risk; HR, hazard ratio.

largest effects have been observed at lag 0-1 days: a 10 μg/m³ increase in PM_{10} was associated with a 0.87% increase (95% CI, 0.34-1.41%) in respiratory mortality. The effects were stronger in females than in males and were also stronger among the elderly than among the young people. The results of sensitivity tests have suggested a linear relationship between daily mortality and PM_{10}. Significant associations of mortality with NO_2 and SO_2 were also observed, with estimated effects even stronger than for PM_{10}. The patterns of NO_2 and SO_2 associations were similar to those of PM_{10} in terms of sex, age, and linearity (45).

The analyses performed by Chen *et al.* on data collected in 16 cities, during the China Air Pollution and Health Effects Study (CAPES, 2001-2008), have confirmed the relationship between PM_{10} and total mortality in the elderly (*Table 2*), with higher effect in 65-year-old people (46). CAPES is the first multi-city study performed in developing countries to analyze the seasonality of PM health impacts. Nation-wide, a 10 μg/m³ increase in the 2-day moving average concentrations of PM_{10} was associated, at lag 0-1, with a significant increase in total mortality of 0.45% in winter and 0.55% in summer. There was no significant effect in spring (increase 0.17%) and fall (increase 0.25%). The seasonal pattern was consistent for both total and cardio-respiratory mortality (33).

Recently, a meta-analysis including 33 studies performed in China has assessed mortality effects of short-term exposure to PM_{10} and $PM_{2.5}$, SO_2, NO_2, O_3, and CO. In general, all considered pollutants were significantly associated with increased mortality risks. In detail, each 10 μg/m³ increase in $PM_{2.5}$ was associated with a 0.51% (95% CI, 0.30-0.73%) increase in respiratory mortality (47).

Bell *at al.* have reviewed all the studies published after 1995 concerning the risk of death or hospitalization by short-term exposure to PM_{10}. They found strong, consistent evidence that the elderly experience higher risk of PM-associated hospitalization and death. Per 10 μg/m³ increase of PM_{10}, meta-analyses showed a statistically higher risk of death of 0.64% (95% CI, 0.50-0.78) for older populations compared to 0.34% (95% CI, 0.25-0.42) for younger populations (48).

Bell *et al.* systematically reviewed also epidemiological evidence, from 1988 to 2013, regarding sensitivity to mortality or hospital admission from short-term O_3 exposure. Through a meta-analysis they found that the strongest evidence for O_3 sensitivity was for age. Per 10-part per billion increase in daily 8-hour O_3 concentration, mortality risk for younger persons (0.60%; 95% CI,

0.40-0.80) was statistically lower than that for older persons (1.27%; 95% CI, 0.76-1.78) (49).

Among a few studies that have assessed the relationships between air pollution and specific respiratory mortality (*Table 2*), Fischer *et al.*, in the Netherlands [1986-1994], found that the daily mortality risk due to COPD, in subjects aged 65 to 74 years, was linked to BS, SO_2, NO_2, and CO with RR ranging from 1.14 to 1.38 (34).

In France, among the elderly people (≥65 years), Filleul *et al.* have estimated an increase in daily respiratory mortality by 9.2%, at lag 0-5 days for a 10 μg/m³ BS increase (35).

Naess *et al.*, in their follow-up study performed in Oslo (Norway, 1992-1998), after adjusting for occupational and educational status, have found significant effects of NO_2, PM_{10} and $PM_{2.5}$ on COPD mortality, particularly in men, among 143,842 persons aged 51 to 90 years (36).

Lastly, Franklin and his colleagues have reported the results of a study carried out in 27 U.S. communities from 1997 to 2002. They observed a positive association between an increase in previous day's $PM_{2.5}$ and respiratory related mortality. The effect was higher in people ≥75 years of age than in those aged <75 years (10).

Long-term effects

Long-term health effects of air pollution have been investigated through longitudinal studies. Chronic exposure to elevated levels of air pollution has been related to the incidence of COPD, chronic bronchitis (CB), asthma, and emphysema (50). There is also growing evidence suggesting adverse effects on lung function related to long-term exposure to ambient air pollution (51).

Long-term morbidity
In Italy, from 1980 to 1993, the Pulmonary Environmental Epidemiology Unit of the CNR Institute of Clinical Physiology (Pisa) performed two large consecutive cross-sectional studies in the rural area of the Po river Delta and in the urban area of Pisa. Prevalence rates of respiratory symptoms, as well as diagnoses of CB, emphysema, asthma, and pleuritis, increased with age and tended to be higher in urban than in rural areas, significantly for cough (37% *vs.* 18%), wheeze (39% *vs.* 27%), and emphysema (22% *vs.* 7%), in males, and for pleuritis (32% *vs.* 18%) in females (52).

In Germany, from 1985 to 1994, consecutive cross-sectional studies were performed within the study on the influence of air pollution on lung function, inflammation and aging (SALIA). Schikowski *et al.* studied the adverse

respiratory effects of long-term exposure to air pollution from industrial sources and traffic in 4,757 women with 55 years of age. Women living near a major road, compared to those living farther away, reported more frequently cough and COPD. An increase in 5-year means of PM_{10} enhanced the risk of COPD. A similar increase in NO_2 was linked to higher risk of CB and COPD (*Table 1*). Moreover, an increment in PM_{10} exposure decreased FEV_1 and forced vital capacity (FVC) by 5.1% (95% CI, 2.5-7.7) and 3.7% (95% CI, 1.8-5.5), respectively (23).

Recently, within the population-based SALIA 2008-2009 cohort, a sub-sample of 402 elderly women has been selected. The aim was to evaluate the association between long-term exposure to air pollution and local inflammation in the lung. Such a study has concluded that long-term exposures to PM and NO_2 from traffic and industry are associated with inflammatory markers in exhaled breath condensate and in induced sputum (53).

Bentayeb and colleagues, in 2104 elderly (≥65 years) living in Bordeaux (France), after adjustment for potential confounders, have reported exposure to elevated mean annual levels (> third quartile) of PM_{10} and SO_2 to be associated with increased prevalence of cough; SO_2 was also positively associated with usual phlegm (*Table 1*). The effects appeared to be stronger for women than for men. Similar results were found when considering 3-year concentrations of proximity air pollutants, thus supporting the hypothesis of a long-term effect of air pollution on respiratory health in the elderly. In this study, it was used a dispersion model taking into account background air pollution, traffic density, meteorological conditions, and topographic conditions. Interestingly, the results have shown that background air pollution underestimates levels of outdoor air pollutants measured at proximity of the residences (24).

With regards to pulmonary function, a longitudinal, population-based prospective study of adults aged ≥65 years living in U.S. [1989-1997] has shown an association between cumulative O_3 or PM_{10} exposure and reduced lung function (6).

A recent longitudinal study in Japan has evaluated long-term impact of air pollution on lifetime non-smoker elderly subjects (≥65 years) who reported pollution-related illness. Further, the study found that normal lung function in the subjects was not restored even after improvement of air pollution (as a result of anti-pollution measures) (54).

Long-term mortality

Air pollution–mortality risk estimates are generally higher for long-term than short-term exposures (55). Indeed, few studies have assessed long-term mortality in the elderly (14).

In 1987, the Netherlands Cohort Study (NLCS) on diet and cancer involved about 120,000 subjects with 55 to 69 years of age. Hoek *et al.* examined the relationship between long-term exposure to traffic-related air pollution, as indicated by BS and NO_2, and mortality (from 1986 to 1994) in a sub-sample of about 5,000 elderly people of the NLCS cohort. Living in close proximity to a major road represented a risk factor for all-cause mortality and cardiopulmonary mortality (56).

Further, Brunekreef *et al.* examined data of the full cohort of NLCS, followed for 10 years (NLCS-AIR, 1987-1996). Information on residential history was used to generate indicators of long-term exposure to air pollutants. All variables representing air pollution and traffic intensity (BS, NO_2, $PM_{2.5}$) were associated with respiratory mortality, with some of the associations being statistically significant (*Table 2*) (37).

Jerrett *et al.* evaluated in an 18-year follow-up [1982-2000] the risk of cardiopulmonary and respiratory mortality associated with long-term exposure to O_3 and PM_{10}. The study involved about 450,000 adults (45 years of age or older) living in U.S. An elevation in O_3 concentration was associated with a significant increase in the risk of death from respiratory causes in both the whole sample and the persons aged >65 years (older people accounting for 20% of the total population) (*Table 2*) (38).

The study of Dong *et al.* on a cohort of about 13,000 adults, followed from 1998 to 2009, provides support to the link between long-term exposure to ambient air pollution and increased risk of mortality from respiratory disease in China. In people 60 years of age or older, the associations were particularly strong for NO_2 and PM_{10} (*Table 2*) (39).

In their prospective cohort study [1990-2006] of 71,431 middle-aged Chinese men, Zhou *et al.* found significant positive associations between PM_{10} levels and the risk of mortality from total, cardiovascular and respiratory mortality. With regard to respiratory mortality, for each 10 $\mu g/m^3$ increase in PM_{10} the risk increased by 1.7% (95% CI, 0.3-3.2) (57).

Overall evidence and conclusions

Figure 1 shows a summary of main health effects caused by common outdoor pollutants in the elderly. Most data have shown higher risks in the elderly compared to the rest of the population. Increased pollution exposures have been associated with increased mortality for cardio-pulmonary or respiratory causes (mainly COPD and

	PM₁₀	PM₂.₅	NO₂	SO₂	CO	O₃	BS
Mortality (cardiopulmonary, respiratory)	X	X	X			X	X
Mortality for COPD	X	X	X	X	X		X
Mortality for pneumonia	X		X	X	X	X	
Hospital admission for respiratory diseases	X		X		X		
Hospital admission for asthma and COPD	X	X	X	X	X	X	
Hospital admission for pneumonia	X	X		X	X	X	
Respiratory symptoms	X		X	X			
Incident COPD	X		X				
Visits for respiratory exacerbation	X	X	X				

Figure 1 Main respiratory health effects due to outdoor pollution exposure in elderly. CO, carbon monoxide; NO₂, nitrogen dioxide; PM, particulate matter; SO₂, sulphur dioxide; O₃, ozone; BS, black smoke; COPD, chronic obstructive pulmonary disease.

pneumonia), with increased number of hospital admissions and emergency-room visits (mainly due to exacerbations of COPD and asthma, or to respiratory tract infections, mainly pneumonia), with higher incidence of respiratory diseases, and with decreased lung function. In general, there is sufficient evidence of the adverse effects related to short-term exposure, while less amount of data are available for long-term effects.

Future epidemiological cohort studies are needed to follow-up the elderly in order to investigate the long-term effects (mainly cardio-pulmonary diseases). This is particularly important for prevention policies, especially in developing countries. Urbanization, the demographic transition from rural to urban location, is associated with shifts from an agriculture-based economy to mass industry, technology, and service. In 2010, for the first time in history, more than 50% of the world's population lived in an urban area. By 2050, 70% of the world's population will be living in towns and cities (58). Urbanization offers opportunities for improvements in population health, but, at the same time, substantial health risks including air pollution.

Outdoor air quality is rapidly deteriorating in major cities in low and middle income countries. With the rapid growth of traffic in developing countries such as China and India, air pollution has outpaced the adoption of tighter vehicle emission standards (59). The above reported population-based, retrospective cohort study conducted in northeast China by Dong et al., over the period 1998-2009, evidenced

increases in the risk of mortality for respiratory disease from ambient air pollution higher than those reported in previous studies conducted in U.S., Europe and Japan (39). The number of deaths due to outdoor air pollution in China rose by about 5%, in India rose by about 12% over the period 2005-2010 (60).

Although rapid economic growth in developing countries has brought many benefits, the adverse health consequences of urbanization pose major policy challenges. In countries changing as rapidly as China or India, frequently updated urbanization data are crucial for health policies to mitigate the adverse health effects, especially in highly vulnerable populations (e.g., older people and children) (61).

Few studies have been directed to elderly people, specifically. Most standardized questionnaires on health assessment were not adapted to the elderly context. As the elderly is a separate group, the implementation of specific methods for this group has to be recommended.

Often, it has not been considered that the relationship between air pollution and respiratory health may be confounded by the presence of comorbidity and treatments, which are frequent in the elderly, as well as by smoking and occupational exposure history, or by genetic factors (62).

Epidemiological studies on adverse effects related to outdoor air pollution in elderly focused mainly on commonly monitored air pollutants, primarily SO₂, CO, NO₂, and PM. Other emerging outdoor air pollutants [e.g., volatile organic compound (VOC), including benzene]

should be also considered.

Moreover, several air pollutants are strongly correlated, and multi-pollutant analyses are needed (63). Data on air pollution exposure are mainly from monitoring stations; to take into account only background air pollution could underestimate the real exposure.

As regard studies on mortality, due to the high presence of comorbidity in the elderly, it would be helpful to perform multi-level studies taking into account not only the underlying cause, but also the other causes of death.

Finally, an emerging problem is the unregulated pollution from ultrafine particles (UFPs, diameter <100 nm). UFPs are ubiquitous and it has been hypothesized that they may have a greater potential for adverse health impacts compared to their larger counterparts, although long-term exposure studies are needed for confirmation. The major source for urban outdoor UFP concentrations is motor traffic. Recent analyses have suggested that the average exposure to outdoor UFPs in Asian cities is about four-times larger than that in European cities (64).

Acknowledgements

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Carcinogenicity of ambient air pollution: use of biomarkers, lessons learnt and future directions

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Abstract: The association between ambient air pollution (AAP) exposure and lung cancer risk has been investigated in prospective studies and the results are generally consistent, indicating that long-term exposure to air pollution can cause lung cancer. Biomarkers can enhance research on the health effects of air pollution by improving exposure assessment, increasing the understanding of mechanisms, and enabling the investigation of individual susceptibility. In this review, we assess DNA adducts as biomarkers of exposure to AAP and early biological effect, and DNA methylation as biomarker of early biological change and discuss critical issues arising from their incorporation in AAP health impact evaluations, such as confounding, individual susceptibilities, timing, intensity and duration of exposure, and investigated tissue. DNA adducts and DNA methylation are treated as paradigms. However, the lessons, learned from their use in the examination of AAP carcinogenicity, can be applied to investigations of other biomarkers involved in AAP carcinogenicity.

Keywords: Carcinogenicity; biomarkers; ambient air pollution (AAP); lung cancer; DNA adducts; DNA methylation

Exposure to air pollution and lung cancer risk

Environmental pollution encompasses a number of hazardous exposures including air, water, and chemical exposures. Ambient (outdoor) and household (indoor) air pollution is a major environmental health risk, affecting populations in developed and developing countries alike (1).

Ambient air pollution (AAP) consists of emissions of complex mixtures of air pollutants from industries, households, cars and trucks (2). Of these pollutants, fine particulate matter (PM) has been widely shown to have adverse effects on human health. Most fine PM comes from fuel combustion, both from mobile sources such as vehicles and from stationary sources such as power plants, industry, households or biomass burning (2). PM can vary in size from ultra-fine particles (UFP) ≤100 nm in diameter, to fine particles ~100 nm -2.5 µm in diameter (PM$_{2.5}$), to larger particles up to 10 µm in diameter (PM$_{10}$) (3), and differential PM sizes can affect pathophysiological pathways independently (3).

On the other hand, household air pollution (HAP) is the result of cooking and heating households using solid fuels (i.e., wood, charcoal, coal, dung, crop wastes) on open fires or traditional stoves. In poorly ventilated dwellings, fine PM concentrations in and around the home can exceed acceptable levels for up to 100-fold (4).

Health risks associated with air pollution include but are not limited to stroke, heart disease, lung cancer, and both chronic and acute respiratory diseases, including asthma (5-8). AAP and its health effects are much more frequently studied compared to HAP.

Of the AAP health effects, lung cancer contributes greatly to air pollution associated mortality. The association between exposure to AAP and lung cancer incidence and/ or mortality has been evaluated in a number of prospective

studies, which are summarized in *Table 1*. Despite that formal statistical significance was not always reached, the evidence linking exposure to urban air pollutants, mainly $PM_{2.5}$ or PM_{10}, and lung cancer is generally consistent. Cohorts from the United States as well as from Europe have found increased risks of lung cancer with higher exposure to PM and other substances present in polluted air, with statistically significant risk ratios (RRs) ranging from 1.14 to 5.21 (*Table 1*).

Based on the available epidemiological and molecular evidence, the International Agency for Research on Cancer (IARC) has recently classified air pollution as Carcinogenic to Humans (Group 1) (37).

Incorporation of biomarkers in measuring exposure and evaluating health effects

Biomarkers were introduced in the study of the carcinogenic effects of AAP under the assumption that they could enhance research on the health effects of air pollution, and other exposures, by improving exposure assessment, increasing the understanding of mechanisms (e.g., by measuring intermediate biomarkers), and enabling the investigation of individual susceptibility.

Biomarkers used in the epidemiology of cancer are usually divided into three categories: markers of internal dose, markers of early response, and markers of susceptibility. In fact, each category includes subcategories. For example, protein adducts and DNA adducts are both markers of internal dose, but their biological significance differs. While protein adducts are not repaired (i.e., they reflect external exposure more faithfully), DNA adducts are influenced by an individual's repair capacity. If DNA adducts are not eliminated by the DNA repair machinery, they induce a mutation. Also, markers of early response are a heterogeneous category that encompasses DNA mutations and gross chromosomal damage. The main advantage of early response markers is that they are more frequent than the disease and can be recognized sooner, thus allowing researchers to identify earlier effects of potentially carcinogenic exposures. Finally, markers of susceptibility include several subcategories; in particular, a type of genetic susceptibility related to the metabolism of carcinogenic substances, and another type related to DNA repair.

Because of their ability to highlight mechanisms, improve exposure assessment, and reflect individual susceptibility, biomarkers have been and will continue to play a vital role in the investigation of the carcinogenicity of AAP.

In this review, we assess DNA adducts as biomarkers of exposure and early biological effect, and DNA methylation as biomarker of early biological change and discuss critical issues arising from their incorporation in health impact evaluations. DNA adducts and DNA methylation are treated here as paradigms, and the lessons learned from their use in the examination of AAP carcinogenicity, can also be applied to investigations of other biomarkers involved in AAP carcinogenicity.

DNA adducts

DNA adducts are covalent bonds arising from the interaction of cancer causing chemicals such as polycyclic aromatic hydrocarbons (PAHs), or metabolites of such chemicals, with sites in DNA (38). Even though adducts can be removed by repair proteins, some can persist, and can contribute to cancer development by causing nucleotide substitutions, deletions and chromosome rearrangements during replication (38).

Several studies have considered DNA adducts as biomarkers of exposure to genotoxic carcinogens, such as PAHs, present in AAP, employing cross-sectional and case-control study designs, some nested within prospective cohorts. Studies which compared the mean DNA adduct levels in individuals with estimated high or low external exposures are summarized in *Table 2*, whereas studies which carried out correlation and regression analyses on all subjects are summarised in *Table 3* (52-66). The majority of studies and two reviews demonstrated positive associations between exposure to air pollution or chemicals in polluted air and the formation of DNA adducts in exposed individuals. Subjects in these studies included a wide range of occupationally and residentially exposed individuals, such as policemen in Bangkok (47), Genova (45), and Prague (49,66), school children in Thailand (50), residents in an industrial area and rural controls in Poland (39), bus and taxi drivers in Stockholm (40), bus drivers in Copenhagen (41), students in Denmark and in Greece (42), as well as street vendors, taxi drivers, gasoline salesmen and road side residents in Benin (51). Only two studies reported no association (54,67).

DNA adducts in children

Fetal exposures and DNA adducts in newborns also showed positive associations (44,53,65). Experimental evidence indicates that developing fetuses are more susceptible than adults to the carcinogenic effects of PAHs. To assess

Table 1 Prospective study results on the relationship between exposure to air pollution and lung cancer incidence and/or mortality, listed by study or cohort

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
American studies							
American legion study							
Buell, 1967 (9)	USA	>10 years in LA county vs. other counties	Lung cancer mortality	Age, sex, smoking, size of birthplace	336,571 person-years	2.5	Not reported
		>10 vs. <10 years in LA county	Lung cancer mortality	Age, sex, smoking, size of birthplace		1.26	Not reported
ASHMOG study							
Mills, 1991 (10)	USA	Total suspended particulate (exceedance frequency of 200 µg/m³)	Cancer in females incidence	Age, sex, education, ex-smoking, ETS, and occupational exposure	6,000	1.72	0.81-3.65
		Ozone (exceedance frequency of 10 pphm)	Lung cancer incidence	Age, sex, education, ex-smoking, ETS, and occupational exposure		2.25	0.96-5.31
Beeson, 1998 (11)	California, USA	Ozone (100 ppb increase)	Lung cancer incidence—males	Pack-year of past cigarette smoking, educational level, and current alcohol use	6,338	3.56	1.35-9.42
		PM₁₀ (IQR increase)	Lung cancer incidence—males	Pack-year of past cigarette smoking, educational level, and current alcohol use		5.21	1.96-13.99
		SO₂ (IQR increases)	Lung cancer incidence—males	Pack-year of past cigarette smoking, educational level, and current alcohol use		2.66	1.62-4.39
		PM₁₀ exceedance frequencies of 50 mg/m³ (IQR increase)	Lung cancer incidence—females	Smoking, age		1.21	0.55-2.66
		PM₁₀ exceedance frequencies of 60 mg/m³ (IQR increase)	Lung cancer incidence—females	Smoking, age		1.25	0.57-2.71
		SO₂ (IQR increases)	Lung cancer incidence—females	Smoking, age		2.14	1.36-3.37

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Abbey, 1999 (12)	USA	PM_{10} (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use	6,338	3.36	1.57-7.19
		PM_{10} (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		1.33	0.60-1.96
		Ozone (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use		2.10	0.99-4.44
		Ozone (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		0.77	0.37-1.61
		SO_2 (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use		1.99	1.24-3.20
		SO_2 (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		3.01	1.88-4.84
		NO_2 (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use		1.82	0.93-3.57
		NO_2 (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		2.81	1.15-6.89
McDonnell, 2000 (13)	USA	$PM_{2.5}$ (IQR increase =24.3 µg/m^3)	Lung cancer mortality		6,338	2.23	0.56-8.94
		$PM_{2.5-10}$ (IQR increase =9.7 µg/m^3)	Lung cancer mortality			1.25	0.63-2.49
		PM_{10} (IQR increase = 29.5 µg/m^3)	Lung cancer mortality			1.84	0.59-5.67
American cancer prevention study II							
Pope, 2002 (14)	USA	NO_2 (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, race, smoking, education, marital status, body mass, alcohol consumption, occupation, and diet	409-493 thousand	1.14	1.04-1.23
Jerrett, 2005 (15)	USA	PM_{10} (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, race, education, smoking, marital status, BMI, alcohol consumption, occupational exposure, diet, and other ecological variables	22,905	1.2	0.79-1.82

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
		Ozone (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, race, education, smoking, marital status, BMI, alcohol consumption, occupational exposure, diet, and other ecological variables		0.99	0.91-1.07
		Distance to freeways (<500 vs. >500 m)	Lung cancer mortality	Age, sex, race, education, smoking, marital status, BMI, alcohol consumption, occupational exposure, diet, and other ecological variables		1.44	0.94-2.21
Turner, 2011 (16)	USA	PM$_{2.5}$ (10 mg/m^3 increase) ACP	Lung cancer mortality	Age, sex, smoking, educational attainment, BMI, chronic lung disease	188,699	NA	1.15-1.27
Pope, 2011 (6)	USA	PM$_{2.5}$ (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, education, marital status, body mass, alcohol consumption, occupational exposures, smoking duration, and diet	1.2 million	1.14	1.04-1.23
Harvard six cities study							
Dockery, 1993 (17)	USA	Inhalable particles: Most polluted vs. least polluted city	Lung cancer mortality	Age, sex, smoking, education, and BMI	8,111	1.27	1.08-1.48
		Fine particles: most polluted vs. least polluted city	Lung cancer mortality	Age, sex, smoking, education, and BMI		1.26	1.08-1.47
		Sulphate particles: most polluted vs. least polluted city	Lung cancer mortality	Age, sex, smoking, education, and BMI		1.26	1.08-1.47
Krewski, 2005 (18)	USA	PM$_{2.5}$ (most vs. least polluted city = 18.6 mg/m^3 increase)	Lung cancer mortality	Age, sex, smoking, education, BMI, diabetes, occupational exposure to dust, gases or fumes	8,111	1.43	0.85-2.41
Laden, 2006 (19)	USA	PM$_{2.5}$	Lung cancer mortality	Age, sex, smoking, education, and BMI	8,096	1.27	0.96-1.69

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Nurses' health study							
Puett, 2014 (20)	USA	72-month average exposures to: PM$_{2.5}$ (for 10 µg/m^3); PM$_{2.5-10}$ (for 10 µg/m^3); PM$_{10}$ (for 10 µg/m^3)	Lung cancer incidence	Cohort restricted to never or quit smoking ≥10 years ago; adjusted for: BMI, alcohol consumption, physical activity, overall diet quality, smoking status (when not stratified by status) and pack-year, months since quitting smoking, secondhand smoke exposure at home, work, and during childhood, and census-tract median home value and median income	1,203,946 person-years	1.37; 1.11; 1.15	1.06-1.77; 0.90-1.37; 1.00-1.32
		Residential distance to major roads per 100 m	Lung cancer incidence	Cohort restricted to never or quit smoking ≥10 years ago Adjusted for: BMI, alcohol consumption, physical activity, overall diet quality, smoking status (when not stratified by status) and pack-years, months since quitting smoking, secondhand smoke exposure at home, work, and during childhood, and census-tract median home value and median income	1,203,946 person-years	0.99	0.95-1.04

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
European studies							
Cohort of Oslo men							
Nafstad, 2003 (21)	Norway	$NO_{(x)}$ (per 10 µg/m^3—home address)	Lung cancer incidence	Age, smoking habits, and length of education	16,209	1.08	1.02-1.15
		SO_2 (per 10 µg/m^3)	Lung cancer incidence	Age, smoking habits, and length of education		1.01	0.94-1.08
French PAARC study							
Filleul, 2005 (22)	France	Total suspended Particulate (exceedance frequency of 200 µg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education	14,284	0.97	0.94-1.01
		Black smoke (for 10 µg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.97	0.93-1.01
		NO (for 10 µg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.97	0.94-1.01
		NO_2 (for 10 µg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.97	0.85-1.10
		SO_2 (for 10 µg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.99	0.92-1.07

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Genair cohort study							
Vineis, 2006 (23)	Ten European countries	PM$_{10}$ (10 mg/m^3 increase)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine	197 cases; 556 controls	0.91	0.70-1.18
		NO$_2$ (10 mg/m^3 increase)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine		1.14	0.78-1.67
		SO$_2$ (10 mg/m^3 increase)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine		1.08	0.89-1.30
		Proximity of residence to major road (exposed *vs.* nonexposed)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine		1.31	0.82-2.09

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Netherlands cohort study on diet and cancer							
Beelen, 2008 (24)	Netherlands	Black smoke concentration	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status	40,114	1.47	1.01-2.16
		Traffic intensity on nearest road	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status		1.11	0.88-1.41
		Living near a major road	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status		1.55	0.98-2.43
Brunekreef, 2009 (25)	Netherlands	Black smoke (per 10 µg/m³)	Lung cancer mortality	Age, sex, smoking status, area-level socioeconomic status	120,000	1.03	0.88-1.20
		Traffic intensity (increase of 10,000 motor vehicles/day)	Lung cancer mortality	Age, sex, smoking status, area-level socioeconomic status		1.07	0.96-1.19
		Black smoke (per 10 µg/m³)	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status		1.47	1.01-2.16
Diet, cancer and health cohort study							
Raaschou-Nielsen, 2011 (26)	Denmark	NOₓ at residence (per 100 µg/m³ increase)	Lung cancer incidence	Age, smoking, ETS, length of school attendance, fruit intake, and employment	52,970	1.09	0.79-1.51
		Traffic load at residence (per 10⁴ vehicle km/day)	Lung cancer incidence	Age, smoking, ETS, length of school attendance, fruit intake, and employment	52,970	1.03	0.90-1.19

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
ESCAPE project—17 European cohorts							
Raaschou-Nielsen, 2013 (27)	Europe	$PM_{2.5}$ (for 10 µg/m³); PM_{10} (for 10 µg/m³)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.18; 1.22	0.96-1.46; 1.03-1.45
		Road traffic within 100 m of the residence: (4,000 vehicle-km per day increase)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.09	0.91.21
		NO_x at residence (per 20 µg/m³ increase)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.01	0.95-1.07
		Traffic intensity on the nearest street (5,000 vehicles per day increase)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.00	0.97-1.04

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Three prospective cohorts							
Raaschou-Nielsen, 2010 (28)	Denmark	NO$_x$ (30-72 vs. <30 μg/m^3)	Lung cancer incidence	Smoking (status, duration, and intensity), educational level, body mass index, and alcohol consumption	679 cases; 3,481 controls	1.30	1.07-1.57
		NO$_x$ (>72 vs. <30 μg/m^3)	Lung cancer incidence	Smoking (status, duration, and intensity), educational level, body mass index, and alcohol consumption		1.45	1.12-1.88
Other studies							
Pope, 1995 (29)	USA	Most vs. least polluted: sulphates	Lung cancer mortality	Smoking	552,138	1.15	1.09-1.22
		Most vs. least polluted: fine particles	Lung cancer mortality	Smoking		1.17	1.09-1.26
Yorifuji, 2010 (30)	Japan	NO$_2$ (10 mg/m^3 increase)	Lung cancer mortality—non smokers	Smoking	14,001	1.3	0.85-1.93
Katanoda, 2011 (31)	Japan	PM$_{2.5}$ (10 mg/m^3 increase)	Lung cancer mortality	Sex, age, smoking status, pack-years, smoking status of family members living together, daily green and yellow vegetable consumption, daily fruit consumption, and use of indoor charcoal or briquette braziers for heating	63,520	1.24	1.12-1.37
		NO$_2$ (10 mg/m^3 increase)	Lung cancer mortality	Sex, age, smoking status, pack-years, smoking status of family members living together, daily green and yellow vegetable consumption, daily fruit consumption, and use of indoor charcoal or briquette braziers for heating	63,520	1.26	1.07-1.48

Table 1 (*continued*)

Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
		SO_2 (10 mg/m³ increase)	Lung cancer mortality	Sex, age, smoking status, pack-years, smoking status of family members living together, daily green and yellow vegetable consumption, daily fruit consumption, and use of indoor charcoal or briquette braziers for heating	63,520	1.17	1.10-1.26
Hales, 2012 (32)	New Zealand	PM_{10} (1 mg/m³ increase)	Lung cancer mortality	Age, sex, ethnicity	50 222	1.015	0.004-1.026
Carey, 2013 (33)	England	$PM_{2.5}$ (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.04	0.99-1.09
		PM_{10} (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.03	0.98-1.08
		NO_2 (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.11	1.05-1.17
		SO_2 (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.03	0.99-1.06
		O_3 (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	0.94	0.90-0.98
Cesaroni, 2013 (34)	Italy	$PM_{2.5}$ (10 mg/m³ increase)	Lung cancer mortality	Sex, marital status, place of birth, education, occupation, and area-based socioeconomic position.	1,265,058	1.05	1.01-1.10
		NO_2	Lung cancer mortality	Sex, marital status, place of birth, education, occupation, and area-based socioeconomic position.	1,265,058	1.04	1.02-1.07
Heinrich, 2013 (35)	Germany	PM_{10} (IQR increase = 7 mg/m³)	Lung cancer mortality	Sex, education, smoking status	4,800	1.84	1.23-2.74
		NO_2 (IQR increase = 16 mg/m³)	Lung cancer mortality	Sex, education, smoking status	4,800	1.46	0.92-2.32
Yorifuji, 2013 (36)	Japan	NO_2 (10 mg/m³)	Lung cancer mortality	Not available	14,001	1.20	1.03-1.40

RR, risk ratio; CI, confidence interval; ETS, environmental tobacco smoke; IQR, interquartile range; PM_{10}, particulate matter with diameter of less than 10 microns; $PM_{2.5}$, particulate matter with diameter of less than 2.5 microns; NO_x, nitrogen oxides with unspecified diameter.

Table 2 Results on the association between air pollution and DNA adducts in exposed individuals; comparison of means analysis (total: 1,044)

First author, Year	Area/country	Exposure	Controlled confounders	Groups, sample size (total: 1,044)	Mean adducts/10^8 nucleotides ± SD (unless otherwise stated)	P
Perera, 1992 (39)	Poland	Environmental air pollution	NA	• Residents in industrial area, 20 • Rural controls, 21	• 30.4±13.5 • 11.01±22.6	<0.05
Hemminki, 1994 (40)	Stockholm, Sweeden	Traffic related air pollution	Age, smoking	• Bus drivers—urban routes, 26 • Bus drivers—sub urban routes, 23 • Taxi drivers—mixed routes, 19 • Controls, 22	• 0.9±0.35 • 1.4±0.48 • 1.6±0.91 • 1.0±0.32	• Non significant • <0.001 • <0.010
Nielsen, 1996 (41)	Denmark	Environmental air pollution	Smoking, PAH[†] rich diet	• Bus drivers in central copenhagen, 49 • Rural controls, 60	• Median: 1.214; range: 0.142-22.24 • Median: 0.074; range: 0.003-8.876	0.001
Nielsen, 1996 [2] (42)	Denmark and Greece	Environmental air pollution	Smoking, sex	• Students in urban universities, 74 • Students in agricultural colleges, 29	• Median: 0.205 • Median: 0.152	0.02
Yang, 1996 (43)	Milan, Italy	Traffic related air pollution	Sex, age, smoking habits	• News stand workers at high traffic areas, 31 • News stand workers at low traffic areas, 22	• 2.2±1.0 • 2.2±1.2	0.27
Topinka, 1997 (44)	Teplice & Prachatice, N&S Bohemia	Residence in industrial area	NA	• Placenta samples—industrial polluted area (winter): GSTM—genotype, 15 • Placenta samples—agricultural area (winter): GSTM—genotype, 17	• 1.49±0.70 • 0.96±0.55	0.027
Merlo, 1997 (45)	Genova, Italy	Ambient PAH concentrations	NA	• Traffic police workers, 94 • Urban residents, 52	• 1.48±1.35 • 1.01±0.63	0.007

Table 2 (*continued*)

Table 2 (*continued*)

First author, Year	Area/country	Exposure	Controlled confounders	Groups, sample size (total: 1,044)	Mean adducts/10^8 nucleotides ± SD (unless otherwise stated)	P
Georgiadis, 2001 (46)	Greece	Environmental Air Pollution	NA	• Students in Athens (highest PAH concentration), 117 • Students in Halkida (lower PAH concentration), 77	• 1.25±1.19 • 1.54±1.19	<0.001
Ruchirawa, 2002 (47)	Bangkok, Thailand	Environmental air pollution	Smoking, sex	• Traffic Policemen, 41 • Office duty policemen, 40	• 1.6±0.9 • 1.2±1.0	0.03
Marczynski, 2005 (48)	Germany	PAH in air (ambient and personal monitoring)	NA[†]	• Samples from 16 workers(increased PAH exposure) • Samples from 16 workers[¥] (reduced PAH exposure)	Range: 0.5–1.19; range: <0.5–0.09	<0.0001
Topinka, 2007 (49)	Prague, Czech Republic	c-PAH (personal exposure)	Smoking, ocuupational duration	• 109 policemen—January (highest exposure) • 109 policemen—March	• 2.08±1.60 • 1.66±0.65	<0.0001
Tuntawiroon, 2007 (50)	Bangkok and Chonburi, Thailand	c-PAH and B[a]P	Age and lifestyle (i.e., ETS, transportation, medication, diet etc.,)	• Bangkok schoolchildren, 115 • Provincial school children (group matching), 69	• 0.45±0.03 • 0.09±0.00	<0.0001
Ayi-Fanou, 2011 (51)	Cotonou, Benin	Environmental air pollution	NA	• Taxi-motorbike drivers, 13 • Intermediate exposure suburban group, 20	• 24.6±6.4 • 2.1±0.6	<0.001
		Environmental air pollution	NA	• Street food vendors, 16 • Intermediate exposure suburban group, 20	• 34.7±9.8 • 2.1±0.6	<0.001
		Environmental air pollution	NA	• Gasoline salesmen, 20 • Intermediate exposure suburban group, 20	• 37.2±8.1 • 2.1±0.6	<0.001
		Environmental air pollution	NA	• Street side residents, 11 • Intermediate exposure suburban group, 20	• 23.78±6.9 • 2.1±0.6	<0.001

NA, not available; PAH, polycyclic aromatic hydrocarbons; c-PAH, carcinogenic polycyclic aromatic hydrocarbons; B[a]P, benzo [a] pyrene; ETS, environmental tobacco smoke; [¥] , the sample sizes reported in the summary tables refer to subjects with measurments available both before and after change in work conditions.

Table 3 Results on the association between air pollution and DNA adducts in exposed individuals; linear regression, logistic regression and correlation analyses

First author, year	Area/country	Exposure	Controlled confounders	Effect measure	Sample size (total: 1,787)	Subject desription	P
Binková, 1995 (52)	Czech Republic	Outdoor air pollution—individual PAH[†]	Age, active and passive smoking, consumption of fried or smoked food, job category	r: 0.541	21	Non smoking women working outdoors up to 8 hours—gardeners or postal workers	0.016
Whyatt, 1998 (53)	Krakow, Poland	Ambient pollution at mother's place of residence	Smoking, dietary PAH, use of coal stoves, home or occupational exposures to PAH & other organics	β: 1.77	19	Mothers not employed away from home	0.05
		Ambient pollution at place of residence	Smoking, dietary PAH, use of coal stoves, home or occupational exposures to PAH and other organics.	β: 1.73	23	Newborns of mothers (high pollution/low pollution group)	0.03
Sørensen, 2003 (54)	Copenhagen	Personal $PM_{2.5}$	Smoking, diet, season	β: -0.0035	75	Students monitored 4 seasons of a year	0.31
Castaño-Vinyals, 2004 (55)	Review	B[a]P (stationary meas.)	Not applicable	r: 0.6	12	Pairs of data	0.038
Peluso, 2005 (56)	10 European countries	O_3 levels	Age, gender, educational level, country and batch	β: 0.066	564	EPIC cohort subjects	0.0095
Neri, 2006 (57)	Review	Environmental pollutants (including ETS exposure)	Not applicable	Not applicable	178	Newborns-17 years old; 2 studies in total—2 with statistically significant results	Not applicable
Pavanello, 2006 (58)	North-East Italy	B[a]P indoor exposure	Smoking, diet, area of residence, traffic near house, outdoor exposure	β: 0.973	457	Municipal workers (non smoking)	0.012
Palli, 2008 (59)	Florence City, Italy	PM_{10} (from high traffic stations)	Smoking	r: 0.562	16	Traffic exposed workers	0.02
Peluso, 2008 (60)	Thailand	Industrial estate residence	Smoking habits, age, gender	OR[†]: 1.65	72; 50	Industrial estate residents control district residents	<0.05
			Smoking habits, age, gender	OR: 1.44	64; 72	PAH exposed workers industrial estate residents	<0.05

Table 3 (*continued*)

Table 3 (*continued*)

First author, year	Area/country	Exposure	Controlled confounders	Effect measure	Sample size (total: 1,787)	Subject desription	P
Pavanello, 2009 (61)	Poland	1-pyrenol	NA	r: 0.67	92	Coke oven workers and controls	<0.0001
Pedersen, 2009 (62)	Copenhagen, Denmark	Residential traffic density	ETS, use of open fireplace, pre-pregnancy weight, folate levels, vitamin B12 levels, maternal education and season of delivery	β: 0.6/0.7	75/69	Women/umbilical cords	<0.01
Eriksen, 2010 (63)	Copenhagen, Denmark	Residence in Copenhagen vs. residence in more rural areas	Years of primary and high school attendence and educational level	• OR: 1.00 • OR: 1.09 • OR: 1.16	115 140 120	• Arhus and neightbouring municipali • Suburban municipalities of Copenhagen • Copenhagen	• 0.27 • 0.08
García-Suástegui, 2011 (64)	Mexico City, Mexico	$PM_{2.5}$	Various risk alleles	r: NR	92	Young adults living in Mexico City	0.013
		PM_{10}	Various risk alleles	r: NR	92	Young adults living in Mexico City	0.035
Herbstman, 2012 (65)	USA	PAH exposure—measured in both air and urine	NA	r: NR	NR	152 participants – prenatal exposure, DNA adducts in cord blood	Not significant
Rossner, 2013 (66)	Czech Republic-Prague	• B[a]P (individual monitors) • B[a]P (stationary meas.) • $PM_{2.5}$ (stationary meas.)	Age, BMI, cotinine, vitamins C, A, E, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides	• β: −0.016 • β: −0.065 • β: −0.003	61 to 65 participants, depending on sampling season	• 0.173 • <0.001 • 0.001
	Czech Republic-Ostrava	• B[a]P (individual monitors) • B[a]P (stationary meas.) • $PM_{2.5}$ (stationary meas.)	Age, BMI, cotinine, vitamins C, A, E, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides	• β: 0.001 • β: −0.002 • β: 0.0	98 to 149 participants, depending on sampling season	• 0.429 • 0.012 • 0.104

r, correlation coefficient; β, linear regression coefficient (change in DNA adduct levels (adducts/10^8 nucleotides) for every unit change in exposure). OR, logistic regression odds ratio; PAH, polycyclic aromatic hydrocarbons; PM_{10}, particulate matter of diameter less than 10 microns; $PM_{2.5}$, particulate matter of diameter less than 2.5 microns; B[a]P, Benzo [a] Pyrene; O_3, ozone; NA, not available; NR, not reported; ETS, environmental tobacco smoke.

fetal versus adult susceptibility to PAHs and second-hand tobacco smoke, a study compared carcinogen-DNA adducts (a biomarker associated with an increased risk of cancer) and cotinine (a biomarker of exposure to tobacco smoke) in paired blood samples collected from mothers and newborns in New York City, USA. The authors enrolled 265 non-smoking African-American and Latina mother–newborn pairs between 1997 and 2001. Despite the estimated 10-fold lower fetal dose, mean levels of B[a]P-DNA adducts were comparable in paired newborn and maternal samples (0.24 adducts per 108 nucleotides in newborns, with 45% of newborns with detectable adducts, *vs.* 0.22 per 108 nucleotides in mothers, with 41% of mothers with detectable adducts). These results indicate an increased susceptibility of the fetus to DNA damage (68).

Dose-response relationship

Lewtas *et al.* [1997] (69) observed that human populations exposed to PAH via air pollution exhibit a nonlinear relationship between levels of exposure and white blood cell-DNA adducts. Among highly exposed subjects, the level of DNA adducts per unit of exposure was significantly lower than those measured after environmental exposures. The observation was confirmed in a meta-analysis of the epidemiological studies (70). The same exposure–dose nonlinearity was observed in lung DNA from rats exposed to PAHs. One interpretation proposed for such an observation is that saturation of metabolic enzymes or induction of DNA repair processes occurs at high levels of exposure (71,72).

DNA methylation

DNA methylation is a biochemical process where a methyl group is added to the cytosine nucleotides mostly found in CpG dinucleotides and this modification influences gene expression (73,74). For example, a high percentage of CpG dinucleotides in repetitive sequences are methylated to inhibit activation and maintain chromosome stability, but CpG sites in CpG islands associated with gene promoters are usually unmethylated. These unmethylated promoter regions allow for active gene transcription (75,76) and also have a role in cell differentiation.

Whole genome methylation is most commonly assessed using surrogate repetitive elements such as long interspersed nuclear element-1 (LINE-1) and Alu repeats (77). Hypomethylation of these endogenous retro-transposons can lead to activation and reposition elsewhere in the genome, causing insertional mutagenesis, transcriptional interference, and genomic instability (77-79). Further to activating repetitive DNA sequences, DNA hypomethylation might also contribute to translocations of these hypomethylated sequences, by loosening chromatin packaging (79-81).

Exposure to AAP, whether short-term or long-term, has been shown to be associated with global hypomethylation. Ten reports (*Table 4*) have recently investigated the effects of AAP exposure on global methylation and a number of them used repetitive elements such as LINE-1, Satα and Alu elements as proxies to whole genome methylation. LINE-1 methylation was frequently found to be altered by exposure to air pollution (82-84). Alu methylation was also significantly altered in three studies (84,85,87) and Satα in one study (88). Lastly, global methylation in healthy adults was decreased following exposure to AAP (86). Despite the small number of available studies, the replication of findings supports AAP's influence on global methylation levels, and these epigenetic changes can contribute to carcinogenesis at least as much as genetic changes.

DNA hypomethylation in children and pre-natal exposures

Two studies investigated global methylation in cord blood and placenta samples and found significant associations with prenatal PAH and PM2.5 exposures (65,89). In addition, when comparing children from the polluted region of Ostrava to children from the non-polluted region of Prachatice, Rossnerova *et al.* [2013] (90) found 9,916 differentially methylated CpGs of which 58 had methylation differences of >10%. All these sites were found to be hypomethylated in Ostrava children demonstrating a significant impact of AAP on the methylation patterns of children.

Critical issues in evaluating the relationship between AAP and biomarkers

Using the pool of evidence on DNA-adducts and DNA methylation as paradigms, a number of critical issues in health impact evaluations using biomarkers arise and several directions for the future of the field can be drawn. The lessons learnt from the experience are critical since the mechanisms through which AAP causes cancer remain to be elucidated and biomarkers of exposure can be incorporated in more accurate exposure assessments.

Table 4 Results on the association between ambient air pollution and Global DNA methylation changes in the cells of exposed individuals

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Baccarelli, 2007 (82)	Boston, USA	Ambient Black Carbon (hourly concentrations measured at a monitoring site approximately 1 km from the site of examination (7-day mean)	LINE-1 methylation	Multiple clinical and environmental covariates	r: –0.11	–0.18, –0.04	718	Subjects from the Normative Aging Study	0.002
		Ambient Black Carbon (hourly concentrations measured at a monitoring site approximately 1 km from the site of examination (7-day mean)	Alu methylation	Multiple clinical and environmental covariates					Not significant
Baccarelli, 2009 (83)	Boston, USA	PM$_{2.5}$ concentrations (7-day mean)	LINE-1 methylation	Age, BMI, cigarette smoking, pack-years, statin use, fasting blood glucose, diabetes mellitus, percent lymphocytes, and neutrophils in differential blood count, day of the week, season, and outdoor temperature	r: –0.13	–0.19, –0.06	718	Subjects from the Normative aging study	<0.001
		PM$_{2.5}$ concentrations (7-day mean)	Alu methylation	Age, BMI, cigarette smoking, pack-years, statin use, fasting blood glucose, diabetes mellitus, percent lymphocytes, and neutrophils in differential blood count, day of the week, season, and outdoor temperature	r: –0.01	–0.07, 0.05			0.71

Table 4 (*continued*)

Table 4 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Tarantini, 2009 (84)	Brescia, Northern Italy	PM_{10} (first day of the week and after 3 days of work)	LINE-1 methylation	Unadjusted	0.02%	SE: 0.11	63	Workers	0.89
		PM_{10} (first day of the week and after 3 days of work)	Alu methylation	Unadjusted	0%	SE: 0.08			0.99
		PM_{10} (first day of the week and after 3 days of work)	iNOS promoter methylation	Unadjusted	−0.61%	SE: 0.26			0.02
		PM_{10} (average level of individual exposure)	LINE-1 methylation	Age, BMI, smoking, number of cigarettes/day	β: −0.34	SE: 0.09			0.04
		PM_{10} (average level of individual exposure)	Alu methylation	Age, BMI, smoking, number of cigarettes/day	β: −0.19	SE: 0.17			0.04
		PM_{10} (average level of individual exposure)	iNOS promoter methylation	Age, BMI, smoking, number of cigarettes/day	β: −0.55	SE: 0.58			0.34
Madrigano, 2011 (85)	New York, USA	$PM_{2.5}$ (IQR increase over a 90-day period)	• LINE1 • Alu	Season, time, smoking, BMI, alcohol intake, medication, batch, % WBC type	• 0.03% • 0.03%	• −0.12, 0.18 • −0.07, 0.13	706	subjects from the Normative Aging Study	• Not significant • Not significant
		Black Carbon (IQR increase over a 90-day period)	• LINE1 • Alu	Season, time, smoking, BMI, alcohol intake, medication, batch, % WBC type	• −0.21% • −0.31%	• −0.50, 0.09 • −0.12, −0.50			• Not significant • <0.05
		SO_4 (IQR increase over a 90-day period)	• LINE1 • Alu	Season, time, smoking, BMI, alcohol intake, medication, batch, % WBC type	• −0.27% • −0.03%	• −0.02, −0.52 • −0.20, 0.13			• <0.05 • Not significant

Table 4 (*continued*)

Table 4 (*continued*)

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
De Prins, 2013 (86)	Belgium	NO$_2$ (IQR increase) 60 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.05	–0.10, –0.01	48	Non-smoking adults	<0.05
		PM$_{2.5}$ (IQR increase) 30 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.06	–0.11, –0.02			<0.01
		NO$_2$ (IQR increase) 60 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.18	–0.37, 0.01			Not significant
		PM$_{2.5}$ (IQR increase) 30 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.14	–0.28, 0.00			<0.05

Table 4 (*continued*)

Table 4 (*continued*)

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Bellavia, 2013 (87)	Toronto, Canada	Fine CAPs for 130 min	LINE1 methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: 0.00	−0.42, 0.44	15	Non-smoking healthy volunteers	Not significant
		Fine CAPs for 130 min	Alu methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: −0.74	−1.18, −0.3			0.0006
		Coarse CAPs for 130 min	LINE1 methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: −0.16	−0.52, 0.24			Not significant
		Coarse CAPs for 130 min	Alu methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: −0.28	−0.65, 0.10			Not significant
Guo, 2014 (88)	Beijing, China	Personal PM$_{2.5}$ (IQR increase)	SATα methylation		−1.35%		120	Truck drivers & office workers	0.01
		Ambient PM$_{10}$ (IQR increase)	SATα methylation		−1.33%				0.01
		Personal PM$_{2.5}$ (IQR increase)	SATα methylation		−2.34%		60	Truck drivers	0.02
		Ambient PM$_{10}$ (IQR increase)	SATα methylation		−1.44%				0.06
		Personal PM$_{2.5}$ (IQR increase)	SATα methylation		−0.95%		60	Office workers	0.26
		Ambient PM$_{10}$ (IQR increase)	SATα methylation		−1.25%				0.12
Herbstman, 2012 (65)	New York, USA	PAH exposure— prenatal	Global Methylation	Ethnicity	β: −0.11	−0.21, 0.00	164	Cord blood samples	0.05

Table 4 (*continued*)

Table 4 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Janssen, 2013 (89)	Belgium	$PM_{2.5}$ (5 µg/m³ increase) Trimester 1	Global Methylation	Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature	−2.13%	−3.71, −0.54	240	Placenta tissue mother-newborn pairs	0.009
		$PM_{2.5}$ (5 µg/m³ increase) Trimester 2	Global Methylation	Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature	−0.43%	−1.81, 0.98			0.55
		$PM_{2.5}$ (5 µg/m³ increase) Trimester 3	Global Methylation	Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature	0.74%	−0.85, 2.33			0.36
Rossnerova, 2013 (90)	Czech Republic	Children from Ostrava (highly polluted) *vs.* Prachatice (control)	27K Methylation: 58 differentially methylated regions	Not available	All sites hypomethylated				<0.05

r, correlation coefficient; β, linear regression coefficient [change in DNA methylation levels (%5mC) per unit change in exposure]; % percent difference; CI, confidence interval; LINE-1, long interspersed nuclear element-1; IQR, interquartile range; PM_{10}, particulate matter with diameter of less than 10 microns; tHcy, total homocysteine; BMI, body mass index; $PM_{2.5}$, particulate matter with diameter of less than 2.5 microns; PAH, polycyclic aromatic hydrocarbons; CAP, concentrated ambient particle.

Confounding

Only 17 of the studies on DNA adducts reviewed here, adjusted for various potential confounders and not all have adjusted for smoking and PAHs in diet, indicating lack of adequate adjustment for confounding. Dietary habits can affect DNA adduct formation, as studies have demonstrated strong negative associations between DNA adducts and consumption of fresh fruit and vegetables, olive oil, and antioxidants as well as positive associations between consumption of charbroiled food and DNA adducts (91,92). In addition, there is almost complete consensus amongst studies in humans, in animals and *in vitro* that smoking, whether active or passive, is associated with DNA adduct formation (93). Also, a recent study has evidenced city-specific spatial and temporal environmental inequalities that relate to the historical socioeconomic make-up of the cities (94). These inequalities become especially important in studies comparing subjects from different cities/rural-urban areas. Considering that PAHs in diet, smoking, exposure to second hand smoke, and socioeconomic status are factors that have an impact on DNA adduct and protein formation, inclusion of these exposures as potential confounders is imperative when investigating the association between exposure to AAP and DNA adducts. It is conceivable, therefore, that the next generation of biomarker studies in relation to AAP could and should address confounding in a more systematic way (e.g., by measuring cotinine as a more accurate reflection of exposure to tobacco). In contrast, almost all studies assessing DNA methylation changes, perhaps due to being more recent, have adequately adjusted for a number of clinical and environmental confounders, including smoking. Further highlighting the inadequate adjustment in the DNA adducts reviewed studies, the confounders considered did not address other carcinogenicity pathways such as inflammation and epigenetics. Considering that these are pathways shown to be influenced by AAP (95-97) and are also shown to be implicated in carcinogenicity (96,98), future studies should use in confounder adjustments markers that are relevant to more than one carcinogenicity pathways.

Reversibility of changes and individual susceptibilities

A second issue that arises from the review of the evidence pool on DNA adducts, is the plasticity and reversibility of the biomarker investigated. Whereas protein adducts cannot be repaired and thus better reflect exposure, DNA adducts can be eliminated by DNA repair mechanisms and are therefore more transient indicators of external exposure. DNA methylation changes have also been shown to be reversible. In addition, other markers of AAP exposure have differential response and step transition times varying at each step with half-lives counted in hours for e.g., 1-hydroxypyrene (1-OHP), oxidized nucleobases, and gene expression, whereas bulky adducts show half-lives of weeks and for chromosomal aberrations (CAs) and micronucei the half-life can be years (95). Hence timing of exposure and the kinetics of the carcinogen and biomarker need to be incorporated in the design of future studies.

In addition, one needs to consider inherited and acquired individual susceptibilities, as DNA adduct levels have been found to be dependent on polymorphisms in metabolic genes involved in adduct formation and DNA repair (i.e., the CYP1A1, MspI, and GSTM1 null genotypes, the XRCC3-241Met homozygote variant allele, and the XPD-Lys751Gln polymorphism with at least 1 variant allele) (99-103). Recently, it was demonstrated the even mitochondrial genetic background can modify the relationship between AAP and biomarkers of inflammation, because of the role of mitochondria in reactive oxygen species production (104). Thus, individual susceptibility can influence different carcinogenicity pathways in different and multi-faceted ways, highlighting the importance of its inclusion in such investigations.

Intensity, duration, and timing of exposure

Furthermore, the issues of intensity, duration, and timing of exposure are of primary importance when evaluating the impact of AAP. As previously discussed, studies show that developing fetuses are more susceptible than adults to the carcinogenic effects of PAHs (44,62,68). Exposure at this critical developmental stage may cause subtle changes that may or may not be repaired. If not repaired, these changes can persist and lead to increased risk of dysfunction and disease later in life (105). Similarly, timing of exposure can be of relevance to other air pollution carcinogenicity biomarkers. For example, exposure to coal and wood smoke after the age of 20 was shown to reduce global DNA methylation levels, but exposure before 20 years was not associated with methylation changes (106).

Studies also show that exposure to PAHs and DNA adduct formation are not linearly associated (69). Instead, among highly exposed subjects the level of DNA adducts per unit of exposure was significantly lower than those at

lower exposures (70).

There is little evidence in the literature about the impact of duration of exposure on the formation of DNA adducts, since no studies have investigated the impact of short-term exposure on DNA adduct formation. However, with respect to mortality, it has been shown that short-term exposure-mortality associations were substantially lower than equivalent long-term associations, a finding which suggests larger, more persistent cumulative effects from long-term exposures (107).

Lastly, the exact composition of AAP exposures needs to be defined in future studies. According to a recently published study, the size fraction of the particles in air are likely to affect different pathophysiological pathways independently (3), therefore AAP exposures with different fractions of differently sized particles might have different biological effects.

In order to add biological credibility and certainty to the impact assessment of AAP, future studies need to aim in bridging current gaps in knowledge about the timing of air pollution effects, the influence of duration of exposure, and the persistence of effects.

Target vs. surrogate tissues

Another important consideration is that most biomarker studies available to date use surrogate tissues, such as blood. AAP is more likely to have the largest impact on sites of deposition where doses are highest, such as the upper aerodigestive tract and lung. If DNA and protein adducts are investigated in target tissues, the associations observed are likely to be much stronger, more reliable, and more accurate. Biomarkers that show great potential for the assessment of AAP exposure and respiratory effects are biomarkers in exhaled breath. Such biomarkers include but are not limited to exhaled nitric oxide (FeNO), exhaled breath condensate (EBC) pH, 8-isoprostane, and interleukin 1β (108,109). These exhaled biomarkers of airway oxidative stress and inflammation can provide a more reliable indication of biologically effective dose with respect to respiratory effects than biomarkers in surrogate tissues (109). Biomarkers relevant to other carcinogenicity mechanisms in exhaled breath remain to be identified.

Measurement error and other biases in study design and analysis

Even though the use of biomarkers can improve exposure assessment in future investigations, the studies included in this manuscript point to an overall need for better and more carefully designed studies to assess the carcinogenicity of AAP. The majority of studies reviewed here used measurements from stationaly air pollution monitoring stations or residence/occupation in a heavily polluted city as proxies to personal exposure to AAP. However, future studies should rely more on individual exposures with the use of mobile, individual sensors, as some of the more recent studies have (66,88). In addition, studies focusing on the comparison of means can only account for a limited number of confounding factors (*Table 2*), introducing bias, and thus more sophisticated statistical analyses should be the preferred in future investigations.

Despite the discussed limitations, DNA adducts and DNA methylation are undeniably valuable biomarkers of exposure and early biological effect regarding AAP. A recent review (95) recognized in addition to DNA adducts and DNA methylation, 1-OHP, CAs, micronuclei (MN), and oxidative damage to nucleobases, as valid biomarkers of exposure to air pollution. These biological markers cover the whole spectrum of progression from external exposure to tumour formation. 1-OHP is an excellent marker of internal dose for PAH exposure, and DNA adducts and oxidized nucleobases are markers of the biologically effective dose, whereas MN, CA, and DNA methylation are good markers of early biological effect (95). DNA adducts and DNA methylation have also been suggested to be predictive for the risk of future cancer (56,98,110,111).

Future directions

Application of DNA adducts and DNA methylation as biomarkers of exposure and early biological effect in large prospective studies on AAP has the potential to reduce measurement error and elucidate possible mechanisms of carcinogenesis. In addition, careful consideration of confounders, use of personal air monitors, investigation of different aetiologically relevant time-windows, and use of target tissues where possible can also improve the quality of future studies thus allowing more weight to be placed on their conclusions. Therefore, high quality prospective population studies can strengthen causality assertions and improve understanding, offering possible avenues through which to combat the problem of AAP carcinogenicity.

The genomics era has led to great improvements in the understanding of cancer biology, and together with the development of high-resolution and high-

throughput technologies interrogating other -omics (such as epigenomics, transcriptomics, proteomics, and metabolomics) they have yielded an unprecedented perspective on cancer omics. These technologies and the emerging knowledge can be used to identify even more biomarkers of AAP exposure and carcinogenicity. Such biomarkers will enable elucidation of new and better understanding of existing carcinogenesis pathways, thus advancing research and addressing the aforementioned gaps in knowledge. Key to such investigations is a multidimensional approach which will help put markers from a specific -omic level into the broader, cellular and molecular context.

Lastly, future studies can be of a transitional nature, aiming to bridge the gap between lab experimentation and population based epidemiology. Validation of *in vitro* results and incorporation of *in vitro* markers in population studies will also strengthen causality inferences, offering multi-level evidence for the carcinogenicity of AAP and the importance of timing, duration, intensity, reversibility of changes and individual susceptibility.

Conclusions

In conclusion, DNA adducts and DNA methylation are important biomarkers that can be used in the investigation of the relationship between AAP and its carcinogenic effects, as they not only improve exposure assessment but also increase our understanding of mechanisms underlying this association. These biomarkers should be used in properly designed future studies of air pollution carcinogenicity. These studies are needed to address current knowledge gaps which would in turn open avenues for prevention, diagnosis, and treatment of cancers and other diseases resulting from air pollution exposure.

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Chinese haze versus Western smog: lessons learned

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Abstract: Air pollution in many Chinese cities has been so severe in recent years that a special terminology, the "Chinese haze", was created to describe China's air quality problem. Historically, the problem of Chinese haze has developed several decades after Western high-income countries have significantly improved their air quality from the smog-laden days in the early- and mid-20th century. Hence it is important to provide a global and historical perspective to help China combat the current air pollution problems. In this regard, this article addresses the followings specific questions: (I) What is the Chinese haze in comparison with the sulfurous (London-type) smog and the photochemical (Los Angeles-type) smog? (II) How does Chinese haze fit into the current trend of global air pollution transition? (III) What are the major mitigation measures that have improved air quality in Western countries? and (IV) What specific recommendations for China can be derived from lessons and experiences from Western countries?

Keywords: Air pollution; emissions; health effects; clean air legislation

History of air pollution and its health consequences

While air pollution in cities has only recently become an alarming concern in China, it has long been recognized as a threat to public health through both its acute and long-term adverse effects. Outdoor or ambient air has always been contaminated with pollutants from natural sources, including, for example, pollens, smoke from fires and volcanoes, and emissions of organic compounds from plants. The current problems and patterns of air pollution date to industrialization and the rise of cities. Together, fossil fuel combustion for heating and cooking and eventually for electric power generation and emissions from factories led to worsening pollution during the Industrial Revolution. The 20th century added mobile sources, including cars, trucks, and other vehicles, as major contributors to urban air pollution.

Urban air pollution was recognized centuries ago as a threat to health. Well-chronicled disasters during the 20th century motivated the actions that have led to marked improvements in air quality in North America and Western Europe. Most notably, the London Fog or the great London smog of 1952, an extreme air pollution event during a week-long episode of atmospheric stagnation, resulted in 10,000 or more excess deaths before the weekly mortality rate returned to the baseline (*Figure 1*) (1). It followed the 1930 episode in the Meuse Valley of Germany (2) and the 1948 episode in Donora, Pennsylvania, in the United States (3); both of these episodes were also accompanied by readily detected excess deaths and were well-documented in the scientific literature. Levels of air pollution during the great London smog were extreme by contemporary standards; "black smoke", a surrogate for airborne particulate matter (PM), reached approximately 4 mg/m^3, orders of magnitude above typical levels in high-income countries of the West. The clear and dramatic loss of lives that was caused by the London smog
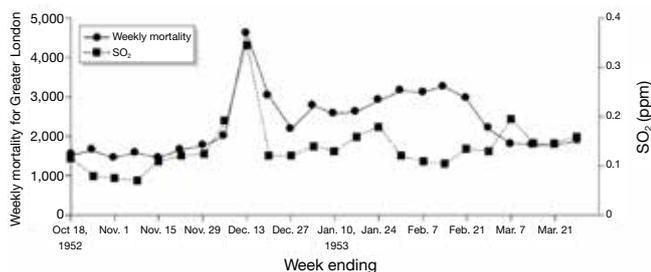
Figure 1 Weekly mortality and SO_2-concentrations for Greater London during the 1952 smog episode. Adapted from Bell and Davis 2001 (1).

of 1952 motivated action: research to better understand the risks of air pollution and regulations to reduce emissions. This research quickly showed that fossil fuel combustion, particularly coal combustion for power generation as well as household space heating, and industrial sources, were resulting in high levels of airborne PM and sulfur oxides.

There were also episodes of serious air pollution with increments in day-to-day mortality counts in the United States. In the 1940s, a new air pollution problem emerged in California—the type of air pollution that was originally referred to as "smog" but more specifically is termed photochemical pollution. The first well-documented episode of such pollution occurred in Los Angeles in 1943. About 10 years later, Dr. Arie Haagen-Smit reported on the nature and causes of photochemical smog, finding that the ultraviolet radiation of sunlight caused photochemical reactions involving nitrogen oxides and hydrocarbons that resulted in an oxidant-rich pollution mixture (4). Ozone is a key component of this mixture and it is used as a general indicator of photochemical smog. Secondary aerosols (e.g., sulfate and nitrate salts), formed through photochemical conversions of organics, sulfur oxides, and nitrogen oxide, are also key components of the complex mixture.

Thus, by the 1960s and 1970s, two broad types of air pollution had been recognized: the mixture of particles and sulfates (acid aerosols) arising from fossil fuel combustion (also called London-type smog) and the photochemical pollution occurring in areas with high vehicle traffic and sunlight (also called Los-Angeles-type smog). The latter type of pollution was initially thought to be a problem localized to California, but with growth of urban regions such pollution was soon present throughout the United States and is now present in many cities around the world. Beyond these two widely occurring air pollution mixtures, other, more specific air pollution problems were identified: carbon monoxide from

combustion sources, lead from lead-containing gasoline and industrial emissions, various metals, carcinogens, and large numbers of air toxics (e.g., benzene and formaldehyde) emitted from industrial and other sources.

These same air pollution problems now affect many of the world's large cities and mega-cities, including those of China. China has rapidly industrialized in the past few decades and added millions of vehicles to its highways, leading to severe decreases in air quality associated with industrial processes, urbanization, and population growth. As such, appropriately, there has been mounting concern on the part of the government and the population of China generally as to the consequences of such high levels of air pollution, which are so high as to greatly limit visibility. Such concerns are warranted as monitoring data and research studies show that much of China now has air pollution sufficiently severe to threaten public health. Indeed, the term '*wu mai*', or 'Chinese haze', is now well known to the general public in China; and $PM_{2.5}$ (particles less than 2.5 micrometers in diameter) is part of everyday conversation among average Chinese people.

This paper provides a snap-shot of China's air pollution problems and describes some key characteristics of the Chinese haze. Furthermore, we offer a perspective on "lessons learned" from research and air quality management in western countries, as, in general, local and regional air pollution has greatly diminished in such countries.

PM₂.₅ in Beijing: a snap shot

Outdoor air pollution was responsible for an estimated 3.3 million premature deaths worldwide in 2010 (5), but the majority of this disease burden occurs in low- and middle-income countries with China contributing 1.2 million deaths to the total. These estimates for the Global Burden of Disease 2010 were based on estimated population-weighted $PM_{2.5}$ concentrations as shown in *Figure 2*.

We now 'zoom in' from this global picture of $PM_{2.5}$ to Beijing's $PM_{2.5}$ levels. While a systematic assessment, considering spatial variations and multiple pollutants, would offer a more complete picture of Beijing's air quality, $PM_{2.5}$ data measured at one site are readily available and provide a clear understanding of PM pollution in Beijing. Consequently, we present data from the "StateAir" website of the U.S. Department of State Air Quality Monitoring. Data from the program (http://stateair.net/web/mission/1/) which tracks daily $PM_{2.5}$ concentrations on the grounds of the U.S. Embassy in Beijing in the winter
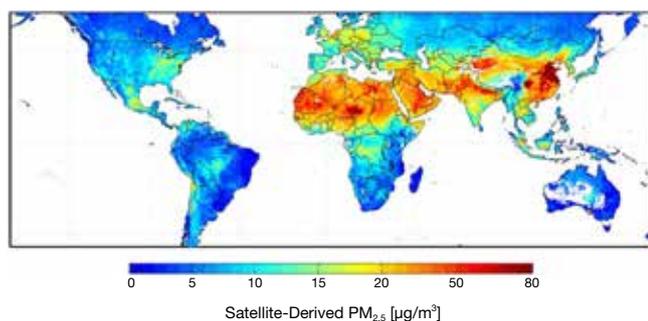
Figure 2 Global distribution of population-weighted $PM_{2.5}$ concentrations. Adapted from van Donkelaar *et al.* (6).
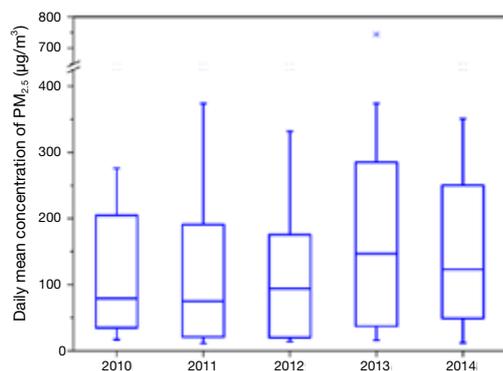

Figure 4 Summer-month $PM_{2.5}$ concentrations measured at the U.S. Embassy in Beijing 2010-2014. Summer months are months of June, July, and August. In these boxplots, the top whisker line corresponds with the 90th percentile, the top line of the box corresponds with the 75th percentile, the middle box line is the median, the bottom box line is the 25th percentile, and the bottom whisker line is the 10th percentile. The dash horizontal line indicates the US Environmental Protection Agency's (EPA) 24-hour standard for $PM_{2.5}$ of 35 µg/m^{3}. Data were retrieved from the Beijing U.S. Embassy air monitoring Twitter feed at https://twitter.com/BeijingAir.

Figure 3 Winter-month $PM_{2.5}$ concentrations measured at the U.S. Embassy in Beijing 2010-2014. Winter months are months of December, January, and February. In these boxplots, the top whisker line corresponds with the 90th percentile, the top line of the box corresponds with the 75th percentile, the middle box line is the median, the bottom box line is the 25th percentile, and the bottom whisker line is the 10th percentile. The star sign is the highest daily concentration. Data were retrieved from the Beijing U.S. Embassy air monitoring Twitter feed at https://twitter.com/BeijingAir.

general, Beijing's cold months are prone to atmospheric inversions, limiting the dispersion and resulting dilution of pollutants emitted at the ground level. In addition, fuel combustion for space heating adds additional $PM_{2.5}$ emissions in the winter months.

In contrast, $PM_{2.5}$ concentrations were generally lower in summer months (see *Figure 4*). But, in 2010 and 2011, approximately half of the days had $PM_{2.5}$ concentrations above 100 µg/m^{3} and even the 10th percentile exceeded the US EPA daily standard of 35 µg/m^{3}.

Chinese haze in the context of global air pollution transition

Over the last four decades, the high-income countries in the West have implemented policies to curtail the severe air pollution events that had plagued them during the early- and mid-20th century. One of the consequences of the increased stringency of pollution control regulations in these countries is the outsourcing of more polluting manufacturing jobs into the low- and middle-income countries by multi-national corporations. In fact, China has been recognized as "the world's factory" in the last few decades. Meanwhile, China has undergone very rapid

months from 2010 to 2014 are shown in *Figure 3*. Daily $PM_{2.5}$ concentrations exceeded 100 µg/m^{3} for more than half of the days and reached as high as 744 µg/m^{3}, more than 20 times the US Environmental Protection Agency's (EPA) 24-hour standard for $PM_{2.5}$ of 35 µg/m^{3}. From 2011 to 2013, median levels appeared to be rising in the same winter months, although the 2014 median level was slighter lower than the 2013 level. Concentrations across all five winters were high, indicating that the severe $PM_{2.5}$ pollution in Beijing was not just associated with one 'bad winter' with unfavorable meteorological conditions for atmospheric dispersion of air pollutants. In
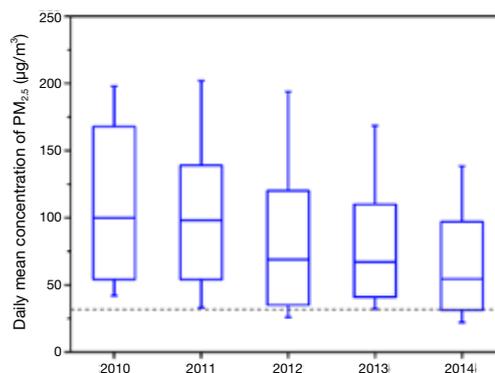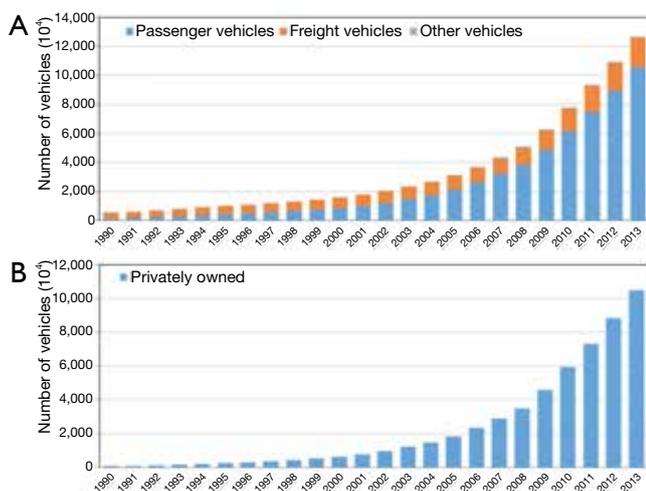
Figure 5 China national number of vehicles from 1990 to 2013, unit: 10,000 vehicles. (A) Blue, orange, and grey bars represent passenger vehicles, freight vehicles and other vehicles, respectively; (B) privately owned vehicles. National Bureau of Statistics of China (http://www.stats.gov.cn/tjsj/).

urbanization and substantial economic growth, as reflected in a double-digit annual increase in GDP for more than a decade until the recent slowing. Increased national wealth has brought higher living standards among the population. For example, the use of cars as a means of personal transportation rose rapidly in China, contributing to growing health concerns related to traffic-related pollution (see *Figure 5*). Consequently, increased vehicle traffic, increased energy use by households, rising factory emissions, and ever-greater numbers of power plants have created high concentrations of air pollutants in the tightly packed population centers. Experience in recent years shows that extremely high concentrations can extend beyond the urban centers in winter months, creating regional pollution problems.

Thus, the Chinese haze is a complex mixture of air pollutants that are present at high concentrations in a city or a region, reflecting a dynamic and growing set of sources. This mixture includes such major components as PM (e.g., PM_{10} and $PM_{2.5}$) and gaseous pollutants (e.g., ozone, nitrogen dioxide, carbon monoxide, and sulfur dioxide). These pollutants are commonly present at elevated concentrations in all polluted atmospheres around the globe. However, the Chinese haze may have particular characteristics compared to outdoor pollution mixtures in Western high-income countries, in addition to the substantially higher $PM_{2.5}$ concentrations in Chinese haze.

In most areas of high-income countries, concentrations of sulfur dioxide (SO_2) and nitrogen dioxide (NO_2), for example, are now quite low; and PM, which has numerous sources, is most intensively investigated and also used as an index for considering the risk posed to public health by air pollution. In contrast, the gaseous pollutants in China are still at concentrations high enough to cause significant health problems, and they remain as useful surrogates for certain air pollution sources (e.g., SO_2 for coal and high-sulfur oil combustion). For example, ambient SO_2 and NO_2 concentrations in Chinese cities have been associated with various adverse health effects, such as pathophysiological biomarkers of adverse cardiopulmonary events (7,8), respiratory symptoms and reduced lung function (9,10), and low birth weight (11). The associations were stronger with SO_2 than $PM_{2.5}$ or PM_{10} in some of these studies.

Sulfur dioxide and main sources

SO_2 has been dramatically reduced in high-income countries by regulations concerning industrial emission controls and other important sources, and reduction of sulfur in fuels. On the whole, global SO_2 emissions have declined from 1990 to 2010 (from 121 to 103 Tg SO_2), led mostly by declines in the developed world (12). However, China alone contributed 29% of global SO_2 emissions in 2011. Emissions of SO_2 peaked in China in 2005, after which the control measures in the 11th Five Year Plan [2006-2010] resulted in a 14% reduction in SO_2 emissions and concomitant 13-15% and 8-10% reductions in ambient SO_2 and particulate sulfate concentrations, respectively, over eastern China (13). Nevertheless, China is still the largest contributor to global SO_2 emissions of any country, largely due to its extensive use of coal for industrial processes and power generation.

In comparison, coal use in the U.S. contributed 6.2% of the global total SO_2 in 2011; and over 70% of total SO_2 emissions in the U.S. came from coal-fired power plants in 2008 (14). However, cleaner coal processing and increased removal of gaseous byproducts from flue gas in the U.S. have reduced SO_2 ambient levels in 2012 and nearly all monitors are below the US EPA one-hour maximum standard of 75 ppb (196 µg/m³) (15).

Nitrogen dioxide and main sources

In many areas of high-income countries, the greatest

source of NO_x (NO_x is the sum of NO_2 and NO but usually dominated by NO_2) is traffic-related pollution. Overall, mobile sources (57.5%), fuel combustion from stationary sources (24.2%), and industrial processes (8.4%) account for the majority of the 14.1 Tg of NO_x emissions in the U.S. (16). In comparison, China is the largest producer of NO_x, emitting 23.4 Tg in 2012. However, 71% was from industrial sources, with motor vehicles only accounting for 27% (17). Beijing has a high concentration of vehicle traffic, but about 50% of the NO_x in its air comes from regional and not local sources (18). The high industrial contribution to Beijing NO_x levels is likely due to sources in the greater Beijing/Tianjin/Hebei area, which accounts for 43% of national coal consumption, 30% of national thermal power consumption, and 50% of national steel and coke production (18).

Because motor vehicles are the major NO_x source in the US and many other countries, the cities with the greatest concentrations of NO_2 in the U.S. often have the worst traffic problems, such as downtown Los Angeles, California (19). As such, great effort has been made to reduce vehicular emissions of NO_x. For example, the State of California has the most stringent vehicular emission standards for NO_2 and other pollutants. The Japanese government enacted the Automobile NO_x Law in 1992 to ban vehicles in certain areas not conforming to emission standards and strengthened it to include PM considerations and stricter standards in 2001. Areas in which the law was enforced had half the average annual NO_2 concentration of unenforced areas (20). Given that regional industrial emissions are the largest NO_x sources in Beijing and likely other Chinese cities, controlling vehicular emissions alone will not be effective in substantially reducing ambient concentrations of NO_2 (and other pollutants).

Clean air legislation in Western countries

Air pollution control strategies have largely reflected a pollution framework that acknowledges PM pollution and photochemical oxidant pollution as the two major threats to public health, while also considering the specific threats outside of this framework that also need to be addressed, such as lead and carbon monoxide. In the U.S., the Clean Air Act, initially passed in 1970, mandated that specific major pollutants, including PM and photochemical oxidant pollution, should be regulated based on the scientific evidence on their harmful effects in humans primarily and to the environment (e.g., crops and atmospheric visibility) secondarily. Six pollutants are currently regulated as so-

called "criteria pollutants" ("criteria" referring to the scientific evidence) and National Ambient Air Quality Standards (NAAQS) for these pollutants are to be set that protect public health with an "adequate margin of safety". For each of the six pollutants, an indicator (e.g., ozone for photochemical oxidant pollution), averaging time (e.g., 24 hours), statistical form (e.g., the 99th percentile), and a level (e.g., 35 μg/m³) are specified; these four elements of the standard are reviewed every five years as evidence accumulates. In the United States, the combination of increasingly strict NAAQS along with components of the Clean Air Act that are directed at particular source categories has proven effective and air pollution has diminished greatly in most places. The regulations have forced new technologies such as emission controls on motor vehicles and scrubbers for sulfur oxides on power plants.

In the United Kingdom, there have also been great improvements in air quality. A somewhat different overall strategy has been followed in comparison with the pollutant-specific approach taken in the United States. Emphasis has been placed on controlling sources and reducing emissions to the extent possible with available technology. The Clean Air Act of 1956 addressed the problem of smoke pollution (London-type smog) and created smoke-free zones to address burning of coal for heating, a potent source of pollution in the densely-settled cities of the United Kingdom. Later Acts addressed industrial sources. Other European nations also had steady improvement in air quality, although there is substantial variation of key indicators across the current European Union. Only now is the European Commission addressing the establishment of uniform air quality standards across its members.

The World Health Organization has developed guidelines for air quality (*Table 1*) (21). These guidelines have no legal basis for implementation by nations, but stand as global guidelines. The guidelines acknowledge the heterogeneity of air pollution across the globe, by not only offering guidelines, but providing targets for those nations, such as China, that cannot feasibly achieve the guidelines at present.

Clean air legislation in China

Ambient air quality has been regulated in China since 1982. In 1996, the standards were strengthened for initially regulated pollutants from the 1982 levels and expanded to include more pollutants under National

Table 1 Sources, health effects, and World Health Organization guidelines on major ambient air pollutants

Pollutant	Source types and major sources	Health effects	WHO guidelines
Particulate matter	Primary and secondary- Anthropogenic: burning of fossil fuel, wood burning, natural sources (e.g., pollen), conversion of precursors (NO_x, SO_x, VOCs) Biogenic: dust storms, forest fires, dirt roads	Respiratory symptoms, decline in lung function, exacerbation of respiratory and cardiovascular disease (e.g., asthma), mortality	PM_{10} Annual mean: 20 $\mu g/m^3$ 24-hour mean: 50 $\mu g/m^3$ $PM_{2.5}$ Annual mean: 10 $\mu g/m^3$ 24-hour mean: 25 $\mu g/m^3$
Ozone	Secondary- Formed through chemical reactions of anthropogenic and biogenic precursors (VOCs and NO_x) in the presence of sunlight	Decreased lung function, increased respiratory symptoms, eye irritation, bronchoconstriction	8-hour mean: 100 $\mu g/m^3$
Nitrogen dioxide	Primary and secondary- Anthropogenic: fossil fuel combustion (vehicles, electric utilities, industry), kerosene heaters Biogenic: biological processes in soil, lightning	Decreased lung function, increased respiratory infection Precursor to ozone. Contributes to PM and acid precipitation	Annual mean: 40 $\mu g/m^3$ 1-hour mean: 200 $\mu g/m^3$
Sulfur dioxide	Primary Anthropogenic: combustion of fossil fuel (power plants), industrial boilers, household coal use, oil refineries Biogenic: decomposition of organic matter, sea spray, volcanic eruptions	Lung impairment, respiratory symptoms. Precursor to PM. Contributes to acid precipitation	Annual mean: 20 $\mu g/m^3$; 10-minute mean: 500 $\mu g/m^3$

Adapted from World Health Organization 2006 (21).

Table 2 Chinese national ambient air quality standards updated in 2012: GB 3095-2012. Grade I standards apply to special areas such as national parks and Grade II standards apply to all other areas

Pollutant (unit)	Averaging time	Standard	
		Grade I	Grade II
SO_2 ($\mu g/m^3$)	Annual	20	60
	24 hours	50	150
	Hourly	150	500
NO_2 ($\mu g/m^3$)	Annual	40	40
	24 hours	80	80
	Hourly	200	200
CO ($\mu g/m^3$)	24 hours	4	4
	Hourly	10	10
O_3 ($\mu g/m^3$)	Daily, 8-hour maximum	100	160
	Hourly	160	200
PM_{10} ($\mu g/m^3$)	Annual	40	70
	24 hours	50	150
$PM_{2.5}$ ($\mu g/m^3$)	Annual	15	35
	24 hours	35	75

Standard GB 3095-1996. However, the standards were revised with less stringent limits for NO_2 and O_3 in 2000. In February 2012, China released a new set of ambient air quality standards, GB 3095-2012. This was the first time that a standard was set for $PM_{2.5}$ in China. The new standards, shown in *Table 2*, will take effect nationwide in 2016, but many cities and regions in China are required to implement the standards earlier than the national timeline (http://datacenter.mep.gov.cn/airdesc.jsp).

Compared to the WHO guidelines (*Table 1*), the Chinese standards (*Table 2*) are substantially less stringent. For example, the 24-hour limits for both $PM_{2.5}$ and PM_{10} in China (Grade II) are three times those recommended by WHO. However, considering the current ambient levels of air pollutants, achieving these relatively non-stringent new standards is still very challenging. Recognizing this challenge, the State Council issued the "Air Pollution Prevention and Control Action Plan" in September 2013, which mandates 25%, 20%, 15%, and 10% $PM_{2.5}$ reductions in the Beijing-Tianjin-Hebei region, the Yangtze River Delta region, the Pearl River Delta region, and all other cities, respectively, by 2017 from their 2012 baseline
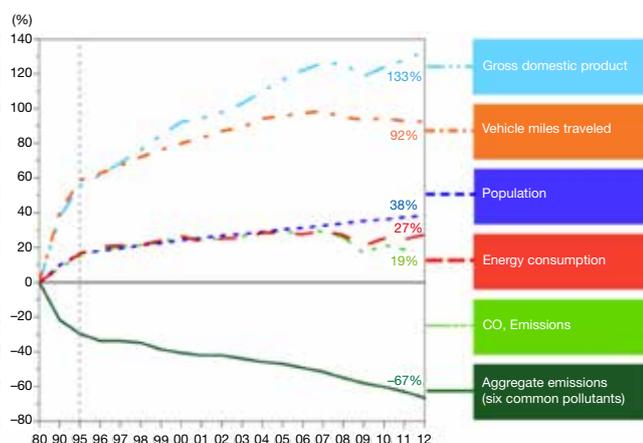
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Figure 6 The 1970 Clean Air Act lowered emissions of key air pollutants even as energy consumption, GDP, vehicle miles, and population increased. This graph was adapted directly from the US EPA website (22).

annual average concentrations. Similarly targets have been or will be set for reducing concentrations of PM_{10} and other pollutants.

On September 9, 2014, the Legislative Affairs Office of China's State Council released the first draft of the highly-anticipated revisions to the national *Air Pollution Prevention and Control Law* and accepted comments on revisions of the law until October 8, 2014. Releasing the draft law for public comment at such an early stage of the process is unusual in China and is a major step forward for both governmental transparency and public participation. This revised draft has been passed by the State Council on November 26, 2014. Upon further revisions, the draft will be submitted to the People' Congress of China that has the ultimate legislative authority.

China's *Air Pollution Prevention and Control Law* was originally enacted in 1987, first revised in 1995, and further strengthened in 2000. However, the earlier versions lacked specific details and mechanisms for enforcement. The most recently revised law incorporates several critical advancements to help address today's severe air pollution problems – the Chinese haze, with the following key points among a list of specific action items. (http://transportpolicy.net/index.php?title=China:_Air_Quality_Standards).

❖ The responsibility of governments for environmental protection is clarified, especially with regards to the role of local governments in managing regional air quality. Local officials will be assessed on compliance with air quality targets and the results

will be released to the public.

❖ The air pollutant emissions control system will be improved by increasing the scope of emissions caps, establishing new targets for key emissions controls, and suspending approval for new projects in areas that exceed emissions targets.

❖ Key areas for air pollution prevention and control are identified including strengthening measures to confront pollution from coal, motor vehicles, industries, dust, and other specific sources.

❖ Environmental air quality and pollution source monitoring will be strengthened by organizing a national monitoring network.

❖ A heavy pollution weather monitoring forecast system will be established whereby provincial governments' environmental and meteorological departments will cooperate to forecast heavy pollution days.

Historical lessons and recommendations

Clean air legislation in some western countries, as described above, has proven effective not only in preventing severe air pollution problems, as seen in the early- and mid-20th century, from recurring but also leading to decreased emissions and general and progressive trends of improving air quality, accompanied with apparently substantial health benefits. For example, although gross domestic product (GDP) and vehicle miles traveled increased by 133% and 92%, respectively, from 1980 (ten years after the US Clean Air Act was passed) to 2012 in the U.S., the aggregate emissions of the six criteria pollutants (PM_{10}, SO_2, NO_2, CO, O_3, and lead) decreased by 67% in the same period (see *Figure 6*) (22). The reduction in SO_2 emissions in this period resulted in a 78% reduction in national SO_2 levels (23). An analysis of the relationship between reductions in ambient $PM_{2.5}$ concentrations and increases in life expectancy in U.S. cities suggests that the 1970 Clean Air Act alone may have extended life expectancy by a half-year to a year (24). The additional health impact of the 1990 Clean Air Act Amendments is shown in *Table 3*.

By contrast, air quality in China has actually worsened long after the establishment of national air quality standards. A key issue for China is not a lack of laws or regulations, but the effectiveness of enforcement of the laws (partly due to inadequacies of the law which lacks key details and mechanisms for enforcement). By contrast, the US Clean Air Act contains an extensive series of requirements

Table 3 The 1990 Clean Air Act Amendment improved a number of health outcomes associated with $PM_{2.5}$ and ozone. Numbers are presented as number of cases avoided

Health effect reductions ($PM_{2.5}$ & ozone only)	Pollutant(s)	Year 2010	Year 2020
$PM_{2.5}$ adult mortality	PM	160,000	230,000
$PM_{2.5}$ infant mortality	PM	230	280
Ozone mortality	Ozone	4,300	7,100
Chronic bronchitis	PM	54,000	75,000
Acute bronchitis	PM	130,000	180,000
Acute myocardial infraction	PM	130,000	200,000
Asthma exacerbation	PM	1,700,000	2,400,000
Hospital admissions	PM, ozone	86,000	135,000
Emergency room visits	PM, ozone	86,000	120,000
Restricted activity days	PM, ozone	84,000,000	110,000,000
School loss days	Ozone	3,200,000	5,400,000
Lost work days	PM	13,000,000	17,000,000

This table was adapted directly from (25).

related to implementation and penalties for not attaining the NAAQS. Today, the Chinese haze is often so severe and widespread that schools are closed, people are advised to stay indoors, and they use air filters or purifiers and wear dust masks during heavy episodes. Some are leaving the cities and even the country in search of clean air for their families, particularly for their children.

The general urgency for action is clear, much as with the London smog of 1952 and other air pollution disasters. In fact, given the extremely high levels of air pollution measured at times, there is risk for such dramatic public health disasters in China. As occurred in the United Kingdom and the United States decades ago, urgent and effective action is needed. The growing concerns of the public as pollution becomes an ongoing and daily health risk need to be matched by a strong governmental response. Strong political will is needed and is apparent at China's national leadership level, along with a willingness to pay for the costs of air pollution control. During the Beijing Olympics in 2008 and the most recent APEC meeting (November 2-11, 2014) in Beijing, aggressive control measures implemented to cut industrial and vehicular

emissions resulted in substantial temporary reductions in pollutant levels in Beijing. After weeks of haze episodes in Beijing in October 2014, a new term was invented, namely "the APEC blue", referring to the blue sky air quality during the APEC meeting in early November. While the public enjoyed the temporary relief from breathing highly-polluted air, President Xi hoped and believed the APEC blue would continue through tireless efforts to control air pollution. With much industry state-operated, China is in a position to take quick action. Given the urgency of the situation, China will need to "leapfrog". It can draw on more than a half-century of research and action in the high-income countries and use strategies and control technologies that have proven effective elsewhere. Most importantly, China has opportunities to utilize the most advanced emission control technologies that were nonexistent at mid-20th century when the current high-income countries experienced their worst air pollution problems. Based on the history of Western smog, we specifically recommend the following.

Control industrial emissions especially from coal combustion

Western countries have had a long history of controlling industrial emissions. The core of the success is the implementation of stringent emission standards. For example, the European Union has experienced significant reductions in emissions of industrial air pollutants as a result of its Large Combustion Plant (LCP) Directive, implemented in 2001, which required that new plants follow strict standards and that old plants exhibit significant reductions in criteria pollutants by 2008 (26). From 2007-2009, LCPs reduced emissions of SO_2 by 44%, NO_x by 27%, and dust by 44% (27). In contrast, China's higher contribution of industrial sources to the total pollutant emissions reflects a lack of enforcement of emission standard laws.

Flue gas scrubbers and other technologies have been instrumental in reducing industrial sources of air pollution. The common technologies used to clean flue gases include electrostatic precipitators and fabric filters for reducing PM, flue-gas desulfurization (FGD) for reducing SO_2; flue-gas denitrification for reducing NO_x through selective catalytic reduction (SCR) and selective non-catalytic reduction (SNCR), and wet and dry scrubbing, absorbers, flue gas recirculation, for a variety of air pollutants. China has adopted most of these technologies to varying extents, including circulating fluidized beds (CFB), in which a gas or fluid is passed through a high kinetic energy solid-fluid

mixture to increase gas/fluid-solid contact, to increase coal combustion efficiency and reduce emissions (28). The extensive use of FGD systems in coal-fired plants during the 11th Five Year Plan has helped to reduce SO_2 emissions from the 2005 peak. However, the relatively high operating costs of NO_x removal systems, such as SNCR/SCR, have impeded their large-scale installation in China until the 12th Five Year Plan [2011-2015] (28,29). Although China has issued laws requiring the use of these technologies, enforcement appears to be too relaxed, given the numbers of plants and factories above emission standards.

Air pollutant monitoring results ordered by the Ministry of Environmental Protection (MEP) in July 2013 revealed that several large-scale steel factories and thermal power plants were consistently breaching discharge standards. From October to December 2013, a comparison of eight major pollution sources each in Hebei and Shandong showed that NO_x emissions were 30 and 37 times greater, respectively, than eight major sources in Beijing (18). Data show that pollutant emissions are largely from coal combustion that is used to power industrial and power plants. Coal use is extremely high in most industrialized regions such as the Yangtze River Delta region (Jiangsu/Zhejiang/Shanghai). This region consumes more coal than the entire U.S. (30), and consequently, it is the area of China with the greatest discharge intensity (tons per km^2 land area) for SO_2 and NO_x (18). Although China has adopted some technologies to clean flue gas on a case-by-case basis, it needs more stringent legislation and, more importantly, enforcement measures to ensure that these technologies are used across the country.

Improve quality of gasoline and diesel fuels

As China continues to add motor vehicles onto its roads, having a cleaner fleet is essential for controlling mobile source emissions. Achieving this goal requires more efficient engines, emission control devices, and/or higher quality fuels (mainly gasoline and diesel). Since 2000, China has adopted increasingly more stringent vehicle emission standards on an incremental basis. The adoption of each set of the standards (e.g., China I, II, and III) generally began in the large metropolitan areas, such as Beijing, Shanghai, and Guangzhou, before proliferating to the rest of country. For example, Beijing implemented Euro 4 standards for light-duty vehicles in 2008 (the year of the Beijing Olympics) and Euro 5-based standards from 2013. This change in requirements resulted in a fast

turnover of the vehicle fleet (as vehicles that cannot meet the standards are removed from the roads). Given that the vehicle fleet in China, especially in metropolitan areas, has already and will likely continue to use the most advanced engine technologies, we think improving fuel quality to further reduce pollutant emissions should be given emphasis at present.

Worldwide, various regulations have phased out harmful additives (e.g., lead) and reduced levels of certain components (e.g., sulfur) in gasoline and diesel. One of the first initiatives to improve gasoline quality was the international effort to phase out tetraethyl lead (TEL) as an anti-knocking agent in gasoline, not only because lead is toxic to humans but also because lead in gasoline can inactivates catalytic converters. Catalytic converters have been used starting in 1975 in the U.S. to catalyze redox reactions that decrease hydrocarbon, CO, and NO_x emissions. Japan enacted the first ban on leaded gasoline in 1986, and most countries including China have officially phased out TEL use by the early 2000s. However, there is evidence of illegal leaded gasoline production in some nations, such as China, despite national regulations (31).

High sulfur content of gasoline and diesel leads to high emissions of sulfur oxides (SO_x, mainly SO_2) and PM. When sulfur in fuels is converted to SO_x during combustion, SO_2 can adsorb onto palladium, platinum, or rhodium catalytic converters (32). This adsorbed sulfur both physically and electrically blocks the binding of other emission chemicals to the catalytic surface, thereby reducing the efficiency of catalytic convertors.

The most stringent sulfur standards for gasoline and diesel (below 15 ppm for diesel) are mostly in the wealthier nations, particularly those in Europe, North America, and Australia (33). Moderate to high diesel sulfur standards (between 50 and 500 ppm) exist in Mexico, many parts of Africa, Russia, South Asia, East Asia, and Southeast Asia. The least stringent standards (greater than 500 ppm) predominate in South America, Central Asia, the Middle East, and most of Africa. By the end of 2014, China will have implemented the China IV sulfur content standard of 50 ppm in both gasoline (GB 17930-2011) and automobile diesel (GB 19147-2013) (http://transportpolicy.net/index.php?title=China:_Fuels:_Diesel_and_Gasoline). Off-road or "general" diesel currently has a standard of 350 ppm (GB 252-2011). By December 31, 2017, implementation of the China V gasoline and diesel standard of 10 ppm sulfur content is planned. However, enforcement remains the key issue for these policy mandates.

Conduct scientific research to support evidence-based policies

Scientific research has been the backbone for evidence-based policies that are designed and implemented to control air pollution in western high-income countries. For example, credible and impartial findings from air pollution health effects studies have helped governments to establish air quality standards; and source apportionment studies have helped to prioritize control targets. In addition to providing research funding, the western governments have made air pollution data (and health data) available to the public, so that the data can be readily used in numerous studies. Until recently, air quality data measured by the government were not available to the public, even though a comprehensive air quality monitoring network across the country has been in place for decades. The government should make further efforts to ensure the transparency and credibility of the monitoring network and to generate data with sufficient quality for scientific research (e.g., not just a gross air quality index or blue sky day count but actual pollutant concentrations). Having such data would not only support research but also enforcement.

At the present time when air pollutant concentrations are so high in China, studies on adverse health consequences may not be necessary to support setting air quality standards (as opposed to such research needs in western high-income countries). However, we recommend planning a national research agenda on air pollution that will address gaps in knowledge that are particular to China and relevant to decision-making. For example, what additional types of studies are needed to characterize the air pollution mixtures in major Chinese cities? What are the long-term and short-term policy options to improve air quality? What kind of interventions at the personal, community, city, and national level can be used to reduce the harmful effects of air pollution? Which subgroups of the general population (e.g., children, people with chronic disease) are most susceptible to air pollution and hence need to be specially protected? How effective is the newly revised Air Pollution Prevention and Control law in reducing ambient concentrations of regulated pollutants and in improving public health?

In summary, we believe China can learn important lessons from the history of air pollution in western high-income countries. The lessons span across science, technology, and policy. A key factor leading to improved air quality is the effective implementation of evidence-based policies. Today's China faces severe air pollution problems similar to the problems that the western countries faced in early and mid-20th century. However, today's China does not need to rediscover all the scientific knowledge and reinvent all the technologies to resolve its problems. We acknowledge that there are unique characteristics and new challenges about the Chinese haze that need to be addressed in terms of both scientific research and policies; but there is a strong and useful body of evidence from research in other countries. With the top Chinese officials showing a strong political will and the general public calling loudly for better air quality, it is the time to use strong science, technology, and law enforcement to win the war against air pollution. (Premier Keqiang Li eloquently declared a war against air pollution in early 2014).

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Chronic effects of air pollution on respiratory health in Southern California children: findings from the Southern California Children's Health Study

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Abstract: Outdoor air pollution is one of the leading contributors to adverse respiratory health outcomes in urban areas around the world. Children are highly sensitive to the adverse effects of air pollution due to their rapidly growing lungs, incomplete immune and metabolic functions, patterns of ventilation and high levels of outdoor activity. The Children's Health Study (CHS) is a continuing series of longitudinal studies that first began in 1993 and has focused on demonstrating the chronic impacts of air pollution on respiratory illnesses from early childhood through adolescence. A large body of evidence from the CHS has documented that exposures to both regional ambient air and traffic-related pollutants are associated with increased asthma prevalence, new-onset asthma, risk of bronchitis and wheezing, deficits of lung function growth, and airway inflammation. These associations may be modulated by key genes involved in oxidative-nitrosative stress pathways via gene-environment interactions. Despite successful efforts to reduce pollution over the past 40 years, air pollution at the current levels still brings many challenges to public health. To further ameliorate adverse health effects attributable to air pollution, many more toxic pollutants may require regulation and control of motor vehicle emissions and other combustion sources may need to be strengthened. Individual interventions based on personal susceptibility may be needed to protect children's health while control measures are being implemented.

Keywords: Air pollution; traffic pollution; asthma; genetic susceptibility; respiratory disease

Introduction

Adverse health effects of exposures to regulated air pollutants have been widely studied (1-6). Other pollutants including volatile organic compounds and ultrafine particles may also have adverse health effects (7), but these pollutants have not been as extensively studied. There is growing evidence for adverse respiratory health effects from ambient air pollutants and near-source local air pollutants such as automobile tailpipe emissions, a major source of air pollution in Southern California and many regions in the world. Recent studies have shown that both ambient air pollutants and near source exposure to traffic-related pollutants are associated with increased incidence of asthma (8-11), lung function deficits (12-14), and airway inflammation (15,16). Traffic-related combustion tailpipe emissions contain high concentrations of reactive gases and high concentrations of ultrafine particles among other toxic compounds. It should be noted that levels of the most toxic of these combustion products are not regulated in the current criteria framework, although the regulated ambient air pollutant levels decreased over the last decades (17).

Because children are more sensitive to the effects of

Table 1 Data collection variations among different cohorts in the Children's Health Study

Variables	Cohort A	Cohort B	Cohort C	Cohort D	Cohort E
Year start	1993	1993	1993	1996	2002
Year end	1995	1998	2001	2004	2016
No. of subjects	938	937	1,806	2,081	5,603
School grade	10	7	4	4	K
Communities*	town code: 1-12				town code: 1-3, 6-9, 11-16
Lung function	All years	All years	All years	All years	Starting from 2007
Fe_{NO}	N/A	N/A	N/A	N/A	Starting from 2005

*Town names represented by the town code: 1, Alpine; 2, Lake Elsinore; 3, Lake Gregory; 4, Lancaster; 5, Lompoc; 6, Long Beach; 7, Mira Loma; 8, Riverside; 9, San Dimas; 10, Atascadero; 11, Santa Maria; 12, Upland; 13, Glendora; 14, Anaheim; 15, San Bernardino; 16, Santa Barbara.

air pollution than adults, due to rapidly growing and developing lungs and immune systems, research about the long-term impact of air pollution on the growth of lung function and respiratory illnesses is important to guide air pollution regulation and early prevention of respiratory diseases in the future. The Children's Health Study (CHS) is one of the largest and most comprehensive investigations of the long-term consequences of air pollution on the respiratory health of children. The CHS has also studied the effects of air pollution on genetic and epigenetic variations in genes in oxidative/nitrosative stress pathway, and how the genetic and epigenetic variations in this pathway influence respiratory health outcomes. Results from the CHS have shown that both ambient air pollution (8,14,16,18-22) and traffic-related pollution (3,9,13,15) have adverse health effects. Additionally, children's vulnerability to air pollution may be increased by higher level of parental stress (23), inadequate antioxidant defenses including low levels of vitamins A and C (24), and variations in the expression or function of antioxidant and inflammatory genes, such as glutathione-S-transferases (*GSTs*) (25-27), arginases (*ARG1* and *ARG2*) (28), and tumor necrosis factor-α (*TNF-α*) (29). In addition to the findings reviewed in 2003 (30), we will summarize more recent findings from the CHS to highlight the heavy burden to children's respiratory health of current air pollution levels, even though these levels are often below national air quality standard.

The Children's Health Study (CHS)

The CHS study design has been described in detail in previous publications (13,30-33). Briefly, more than 11,000 school children were selected from classrooms in

16 communities in multiple waves of subject recruitment starting in 1993 to maximize the differences in regional air pollution concentrations and mixtures (*Table 1*). Beginning from study entry and continuing until high school graduation, yearly questionnaires assessed the development of respiratory symptoms and current activity patterns. Lung function was measured annually through spirometry. School absences were actively ascertained to evaluate the effects of pollution on acute respiratory illnesses. Outdoor concentrations of ozone (O_3), particulate matter (PM) of less than 2.5 μm and less than 10 μm aerodynamic diameter ($PM_{2.5}$ and PM_{10}, respectively), and nitrogen dioxide (NO_2) were measured continuously at central monitoring stations within each community. Several metrics of traffic-related pollution have been used, including (I) proximity of the residence to the nearest freeway or roadway; (II) average number of vehicles traveling within 150 m of the residence each day; (III) model-based estimates of traffic-related air pollution at the residence or school derived from dispersion models (CALINE) (8,12,34) and land-use regression exposures models (13).

For the cohort of kindergarten and first grade student recruited in 2003 to study the relationship between air pollution and airway inflammation (Cohort E, *Table 1*), exhaled nitric oxide (Fe_{NO}) was collected using both an offline breath collection technique according to American Thoracic Society (ATS) guidelines [ATS 1999; ATS/European Respiratory Society (ATS/ERS) 2005] in the initial years of the study, and an online Fe_{NO} collection in subsequent study years (32).

Participants provided DNA beginning in 1998 using standard buccal cell collection procedures (35). Genomic DNA was isolated using a Puregene DNA isolation kit

(Gentra Systems, Minneapolis, MN). Each genotype was validated by using PCR/restriction fragment length polymorphism methods (36). A nested case-control sample of 769 asthmatics and 1007 controls, who were either Hispanic white (n=817) or non-Hispanic white (n=959), were selected into CHS genome-wide association study. The genotyping was performed at the USC Epigenome Center using the Illumina HumanHap550, HunmanHap550-Duo or Human610-Quad BeadChip microarrays.

Main findings

Air pollution associations with asthma occurrence

Ambient air pollution has been associated with asthma prevalence and incidence in the CHS. In Cohort C,D, higher local NO_2 concentrations were associated with higher asthma prevalence [odds ratio (OR), 1.83; 95% confidence interval (CI): 1.04-3.22; per interquartile range (IQR) =5.7 ppb NO_2] after adjusting for sex, race, Hispanic ethnicity, cohort, and community (8) and higher risk of new-onset asthma [hazard ratio (HR), 1.29; 95% CI: 1.07-1.56; per IQR of 6.2 ppb NO_2] after adjusting for Hispanic ethnicity, medical insurance, cohort, community, and relative humidity (9). In Cohort A-D, Regional O_3 was associated with asthma incidence, but this association was modified by exercise (11). Specifically, the relative risk of asthma incidence associated with high regional O_3 was three times higher among children playing three or more team sports, compared to children playing no sports after adjusting for ethnicity and community with baseline strata for age and sex (OR, 3.3; 95% CI: 1.9-5.8). A statistically significant positive association between number of team sports played and asthma incidence was observed only in communities with high O_3 (means of O_3 concentrations in high and low pollution communities =59.6 ppb and 40.0 ppb, respectively) (OR, 1.4; 95% CI: 1.0-2.1). However, in the subsequent Cohort E, regional NO_2 and O_3 did not appear to be associated with asthma incidence after additionally adjusting for non-freeway traffic-related pollution at home and school (HR, 1.37; 95% CI: 0.69-2.71; and HR, 1.01; 95% CI: 0.49-2.11, respectively) (10).

Across CHS cohorts, several metrics of traffic-related pollution have shown adverse effects on asthma prevalence and incidence, independent of regional ambient air pollution. In Cohort C and D, the risk of life-time asthma was 1.9-fold among children with closer residential distance to a freeway (below 25th percentile) compared to children with farther residential distance from a freeway (above 75th percentile) after adjusting for sex, race, Hispanic ethnicity, cohort, and community (OR, 1.89; 95% CI: 1.19-3.02) (8). An IQR [2.3 ppb nitrogen oxide (NO_x)] increase of CALINE estimated freeway-related NO_x was associated with more than 2-fold increased risk of lifetime asthma (OR, 2.22; 95% CI: 1.36-3.63) (8). In Cohort A-D, children with residences within 75 m of a major roadway had a 29% increased risk of lifetime asthma and a 50% increased risk of prevalent asthma after adjusting for age, sex, race community, and language of the questionnaire (English/Spanish) (3). In Cohort E, after adjustment for race/ethnicity and for baseline hazards strata of age at study entry and sex, and random effects of school and community, an IQR (8 ppb NO_x) increase in CALINE estimated non-freeway traffic-related pollutions near home and school were both associated with a 1.5-fold increased risk of new-onset asthma, and these results were robust to adjustment for ambient NO_2 (OR, 1.46; 95% CI: 1.16-1.84) for home; and OR, 1.45; 95% CI: 1.03-2.06 for school) (10). Recent results further suggested that the effect of traffic-related pollution on the risk of new-onset asthma can be modified by parental stress levels. After adjusting for race/ethnicity and community with baseline strata for age and sex, an IQR increase of non-freeway traffic-related pollution (21 ppb of NO_x) was associated with a 1.5 times (HR, 1.51; 95% CI: 1.16-1.96) higher hazard of incident asthma for children with high parental stress versus a 1.1 times (HR, 1.05; 95% CI: 0.74-1.49) higher hazard of incident asthma for children with low parental stress (23), where parental stress was assessed by perceived stress scale (PSS >4) (37). These results from the CHS are consistent with a growing body of evidence from international studies indicating that that exposure to vehicle emissions increases the risk of new-onset asthma (4,38,39).

Air pollution effects on children with asthma

Air pollution may play a role in the exacerbation of existing asthma. In the CHS, children with physician-diagnosed asthma had more chronic lower respiratory tract symptoms including bronchitis and phlegm production if they lived in communities with higher levels of NO_2, PM_{10}, and $PM_{2.5}$ (40). Two pollutant models showed that within-community variations in organic carbon (OC) (OR, 1.41 per ppb; 95% CI: 1.12-1.78) and NO_2 (OR, 1.07 per ppb; 95% CI: 1.02-1.13) had robust positive associations with the risk of bronchitis symptoms after adjusting for age, maternal and

child's smoking history, sex, race, community and other pollutants including PM, O_3, organic and inorganic acid, and elemental carbon (EC) (18).

Air pollution was also associated with acute respiratory symptoms including wheezing and asthma medication use. Amongst fourth-grade school children, an IQR (13.39 μg/m³) increase in monthly average PM_{10} was associated with almost a 3-fold higher monthly prevalence of wheezing during the spring and summer months after adjusting for age, sex, race/ethnicity, community, home characteristics, and secondhand tobacco smoke (OR, 2.91; 95% CI: 1.46-5.80), but this association was not significant during the fall and winter months (19). Pollutants primarily produced by photochemistry were associated with asthma medication use. IQR increases in monthly average O_3 (27.83 ppb), nitric acid (HNO_3) (1.64 ppb), and acetic acid (2.66 ppb) levels were associated with 80% (OR, 1.80; 95% CI: 1.19-2.70), 80% (OR, 1.80; 95% CI: 1.23-2.65) and 60% (OR, 1.57; 95% CI: 1.11-2.21) more monthly prevalence of asthma medication use (19). Associations between air pollutants and asthma medication use were stronger among children who spent more time outdoors (OR, 3.07; 95% CI: 1.61-5.86 for O_3; OR, 1.93; 95% CI: 1.18-3.15 for HNO_3; and OR, 2.38; 95% CI: 1.37-4.14 for acetic acid, respectively), compared to children who spent less time outside. Recent findings suggest that traffic-related pollution was also associated with children's wheezing (41). Among kindergarten and first grade (Cohort E) children aging 4.4- to 8.9-year-old who were diagnosed with asthma, per increase of 9 minutes in school commuting time was significantly associated with 50% increase (OR, 1.54; 95% CI: 1.01-2.36) of prevalence of severe wheezing using the criteria from the International Study of Asthma and Allergies in Childhood (ISAAC) (42) after adjusting for age, sex, race, community, mode of travel to school, and modeled residential traffic-related pollution. This association was more striking among asthmatic children with commuting times 5 minutes or longer (OR, 1.97; 95% CI: 1.02-3.77). Other effects of air pollution on asthmatic children include increased emergency department visits or hospitalizations (43), and higher school absence rates (44).

Taken together, these results from the CHS demonstrate that the effects of ambient air pollution and traffic-related air pollution on childhood asthma pose a large burden to public health and the economy. According to the CHS estimates, the successful improvement in O_3 levels in Southern California during the year 1990 to 1999 reduced more than 2.8 million school absences, which saved more

than \$220 million (45). On the other hand, asthma burden attributable to air pollution in two California communities was \$18 million yearly during 1996 to 2004, and half of this cost was due to traffic-related pollution (46).

Air pollution and lung function

The deficit in the growth of lung function is another chronic health effect of air pollution. Following children from age 10 to 18 years, deficits in the growth of forced expiratory volume in one second (FEV_1) were associated with exposure to higher levels of NO_2, $PM_{2.5}$, EC, and acid vapor after adjusting for sex, Hispanic ethnicity, log-transformed height, BMI, BMI squared, present asthma status, child's smoking history, secondhand tobacco smoke, community, exercise or respiratory tract illness on the day of the test, and indicator variables for field technician (P=0.005, 0.04, 0.007, and 0.004, respectively) (14). Deficits in the growth of forced vital capacity (FVC) were associated with exposure to NO_2 and acid vapor (P=0.05 and 0.03, respectively), and deficits in the growth of maximal midexpiratory flow rate (MMEF) were associated with exposure to NO_2 and EC (P=0.02 and 0.04, respectively). Similar associations were also observed for FEV_1 attained at the age of 18 years (14). For example, the estimated proportion of 18-year-old subjects with a low FEV_1 (defined as a ratio of observed to expected FEV_1 of less than 80%) in the community with highest level of $PM_{2.5}$ was 4 times more than the community with the lowest level of $PM_{2.5}$ (7.9% *vs.* 1.6%, P=0.002).

Exposures to traffic-related pollution were associated with lung development as well. After adjusting for height, height squared, BMI, BMI squared, present asthma status, community, exercise or respiratory illness on the day of the test, any tobacco smoking by the child in the last year and field technician, children who lived within 500 m of a freeway had significant deficits in FEV_1 and MMEF growth from age 10 to 18 compared to children who lived more than 1,500 m from a freeway (P=0.01 and 0.03, respectively) (12). Joint models revealed that adverse effects of traffic exposures on the growth of FEV_1 were independent of regional air pollutions (NO_2, Acid vapor, PM_{10}, $PM_{2.5}$, and EC). In another cross-sectional analysis of children with mean age of 11.2 years, residential proximity to a freeway was shown to be inversely associated with the reduction in FVC after adjusting for log-transformed height and height squared, BMI and BMI squared, age, sex, race/ethnicity, community, respiratory illness on the day of the test and

field technician (13). Living within 500 m of a freeway was associated with 2% deficit in FVC (P=0.009). Additionally, higher model-based estimate of near-roadway (freeways, highways and large surface streets) NO_x was associated with deficits in FEV_1 and FVC (P=0.005 and 0.048, respectively) (13). Consistent with our previous findings, near-roadway NO_x and regional air pollutants (O_3, $PM_{2.5}$, PM_{10} and NO_2) had independent inverse association with deficits in FEV_1 and FVC. There was an evidence that associations between residential near-roadway NO_x and deficits in FEV_1 and FVC might be modified by parental stress (both interaction P<0.01) (47). Significant inverse associations were only observed among children from high-stress households (parental PSS >4) after adjusting for log height and log height squared, BMI and BMI squared, age, sex, race/ethnicity, community, respiratory illness on the day of the test and field technician, but not among children from low-stress households (parental PSS ≤4). However, no interactions were found for air pollution with sex and asthma status.

Air pollution and airway inflammation

Airway inflammation is a potential mechanism underlying the effects of air pollution on asthma exacerbations (48). The exhaled nitric oxide fraction (Fe_{NO}) is a noninvasive marker of aspects of airway inflammation that has been developed and validated in the past decade (49,50). Children with Fe_{NO} in the highest quartile at the start of follow-up (>14.8 ppb at 50 mL/s) had more than a 2-fold increased risk of new-onset asthma compared to children with Fe_{NO} in the lowest quartile (<7.8 ppb at 50 mL/s) after adjusting for race/ethnicity, lifetime wheeze and community with baseline strata for age and sex (HR, 2.1; 95% CI: 1.3-3.5) (51). In the CHS, both regional air pollution and traffic-related pollution were associated with higher Fe_{NO}. Among children ages 7 to 11 years old, daily 24-h cumulative lagged averages of $PM_{2.5}$ (over 1-8 days), PM_{10} (over 1-7 days) and O_3 (over 1-23 days) were significantly associated with 17.4% (P<0.01), 9.3% (P<0.05) and 14.3% (P<0.01) higher Fe_{NO} levels over the IQR (7.5 µg/m³, 12.97 µg/m³, and 15.42 ppb for $PM_{2.5}$, PM_{10}, and O_3, respectively) of each pollutant, respectively, after adjusting for age, sex, race/ethnicity, community, asthma, asthma medication use, history of respiratory allergy, time of Fe_{NO} collection, BMI, secondhand tobacco smoke, parental education, language of the questionnaire (English/ Spanish), season and whether Fe_{NO} testing was conducted

outdoors (16). These associations did not significantly vary by sex, asthma, and respiratory allergy status. However, results suggested that the effects of air pollutants were relatively larger in the warm season compared to the cold season. Longitudinal analysis showed increases of long-term (annual average) exposures of NO_2 and $PM_{2.5}$ (scaled to IQR of 1.8 ppb and 2.4 µg/m³, respectively) were associated with 2.29 ppb (P=0.02) and 4.94 ppb (P=0.005) increase in Fe_{NO} after adjusting for age, sex, race/ethnicity, asthma, asthma-medication use, history of respiratory allergy, day of Fe_{NO} collection, season, and short-term (lags of up to 60 days prior to the day of Fe_{NO} test) effects of the same air pollutant (52).

From a set of traffic-related pollution metrics, only the length of road in a circular buffer around the residence was found to be positively associated with Fe_{NO} (15). This association was restricted to children with asthma, and was strongest in the 50 m buffer, the smallest buffer considered. Specifically, a 100 m increase in the length of road in a 50 m buffer around subject's home was associated with a 46.7% (95% CI: 14.3-88.4%) higher Fe_{NO} in children with asthma and 0.2% lower (95% CI: −5.5-5.3%) Fe_{NO} in children without asthma after adjusting for age, sex, race/ethnicity, community, asthma, asthma-medication use, rhinitis history, BMI percentile, secondhand tobacco smoke, parental education, month and hour of Fe_{NO} collection and outdoor testing. Our future work will investigate the longitudinal relationships between traffic-related pollutions and Fe_{NO}, as well as whether Fe_{NO} influences the relationship between air pollution and asthma incidence.

Genetic susceptibility and gene-environmental interaction

In the past 10 years, the CHS has revealed a great amount of evidence for genetic influence on the association between air pollution and respiratory illness (*Table S1*). The associated genes include GSTs (encoded by *GSTM1*, *GSTP1*, and *GSTT1*), microsomal epoxide hydrolase (*EPHX1*), catalase (*CAT*), myeloperoxidase (*MPO*), heme oxygenase 1 (*HMOX-1*), tumor necrosis factor (*TNF*), arginases (encoded by *ARG1* and *ARG2* genes), inducible nitric oxide synthase (iNOS, encoded by *NOS2*), and transforming growth factor β1 (*TGFβ1*).

Incomplete combustions from smoking and fossil fuels contain high levels of polyaromatic hydrocarbons (PAHs), which can lead to oxidative stress and has been shown to relate to asthma and wheeze (53-55). Thus, genes involved in xenobiotic-induced oxidative stress were of great interest.

GSTM1 null and *GSTP1* (rs1695) A/A genotype were shown to enhance nasal allergic responses with increased IgE levels (56). In the CHS (*Table S1*), *GSTP1* rs1695-G and the upstream promoter single-nucleotide polymorphism (SNP) rs6591255-A allele were both associated with increased occurrences of lifetime asthma and wheezing (25,57). There was a significant interaction between *in utero* exposure to maternal smoking and rs1695 genotype on the association with wheeze (25). Compared to children with no exposure and rs1695 A/A genotype, children exposed to *in utero* maternal smoking and having rs1695 A/G or G/G genotypes had a 2-fold increased risk of early-onset asthma, current wheezing and medication use for wheeze after adjusting for age, sex, ethnicity, community, gestational age, and secondhand tobacco smoke (OR, 2.0; 95% CI: 1.1-3.3; OR, 1.9; 95% CI: 1.3-2.6); and OR, 1.9; 95% CI: 1.2-2.8, respectively). In contrast, children carrying rs1695 A/G or G/G genotypes was found to be associated with 40% lower risk of new onset asthma compared to children with rs1695 A/A genotype after adjusting for ethnicity and community (HR, 0.6; 95% CI: 0.4-0.8) (27). The opposite direction of associations between rs1695 genotype with wheezing and asthma incidence suggests rs1695 might have pleiotropic effect on asthma traits.

EPHX1 is also involved in the xenobiotic metabolism, but less studied. We found *EPHX1* SNPs rs1051740 and rs2234922 were associated with several asthma outcomes (57) (*Table S1*). After adjustment for age, sex, race/ethnicity, *in utero* maternal smoking, number of smokers at home, community, parental education, health insurance and parental history of asthma, children with rs1051740 C/C genotype had a 49% reduced risk of late onset asthma (OR, 0.51; 95% CI: 0.29-0.88) compared to children with T/T genotype. Children with rs2234922 A/G genotype had 42% (OR, 1.42; 95% CI: 1.14-1.76); 45% (OR, 1.45; 95% CI: 1.12-1.89) and 58% (OR, 1.58; 95% CI: 1.19-2.10) increases of lifetime, current and late onset asthma compared to children with A/G genotype. The association between *EPHX1* phenotypes and the risk of asthma varied by the *GSTP1* rs1695 genotype and residential proximity to a major road. Among children with rs1695 G/G genotype, those had high *EPHX1* activity phenotypes were of a 4-fold increased risk of lifetime asthma compared to children with low/intermediate *EPHX1* activity phenotypes (OR, 4.0; 95% CI: 1.97-8.16). This association was not significant among children with rs1695 A/A or A/G genotypes. Association between high *EPHX1* activity and the increased risk of lifetime asthma was also found among children who lived within 75 m of a major road. Children having high *EPHX1* activity phenotype and rs1695 G/G genotype who lived within 75 m of a major road had a 9-fold increased asthma risk compared to those having low/intermediate *EPHX1* activity and rs1695 A/A or A/G genotypes, and living more than 75 m of a major road (OR, 8.91; 95% CI: 2.40-33.12). No significant association was found for children living at least 75 m far from a major road.

For lung function, variation in the GST mu family (*GSTM2-5*) locus was found to be associated with lower FEV_1 and MMEF (26) (*Table S1*). Two haplotypes of *GSTM2* (one showed risk effect and one showed protective effect) were significantly associated with 8-year growth of FEV_1 and FVC after adjusting for height, height squared, BMI, BMI squared, current asthma status, exercise or respiratory illness on the day of the test, any tobacco smoking by the child in the last year, *GSTM1* null genotype, and field technician (all P<0.02). Significant associations were only found among children exposed to *in utero* maternal smoking. One haplotype of *GSTM3* was associated with slower growth of MMEF compared with children with other haplotypes (P=0.002). One haplotype of *GSTM4* was associated with decreased growth in FEV_1 (P=0.01), FVC (P=0.03), and MMEF (P=0.05) from age 10 to 18. For respiratory illness-related absences, minor alleles in SNPs of *GSTP1* including rs6591255-A, rs1695-G, and rs749174-T were associated with a protective effect for respiratory illness-related absences after adjusting for age, sex, race, community, asthma status, family income, health insurance, secondhand tobacco smoke, *in utero* maternal smoking, and BMI (OR, 0.61; 95% CI: 0.43-0.87 for Hispanic White; and OR, 0.86; 95% CI: 0.71-1.04) for non-Hispanic White) (58). Additionally, the protective effect was restricted among children unexposed to *in utero* maternal smoking.

Catalase (encoded by *CAT*), myeloperoxidase (encoded by *MPO*), and heme oxygenase (encoded by *HMOX-1*) are enzymes in the oxidative stress defense pathway (59,60). Among children in the CHS (*Table S1*), we found there was an epistatic interaction of *CAT* (rs1001179) and *MPO* (rs2333227) for their association with respiratory-related school absences after adjusting for age, sex, race, community, family income, health insurance, secondhand tobacco smoke, *in utero* maternal smoking, BMI, cat or dog ownership, and asthma status (61). Children had *CAT* (rs1001179) G/G genotype and at least one A allele of *MPO* (rs2333227) had 35% higher risk of respiratory-related school absences compared to children with *CAT* (rs1001179) G/G genotype and *MPO* (rs2333227) G/G genotype (OR,

1.35; 95% CI: 1.03-1.77). The epistatic interaction was significant among children living in communities with high O_3 level, but was not evident in communities with low O_3 level. The number of $(GT)_n$ repeats of the *HMOX-1* gene showed a bimodal distribution with two peaks being 23 and 30 repeats among Hispanics and non-Hispanic whites (22). Among non-Hispanic whites, children carrying at least one *HMOX-1* "short" alleles (<23 repeats) were associated with 36% lower risk of new-onset asthma compared to children who had no "short" allele controlling for communities with age- and sex-specific baseline hazard (HR, 0.64; 95% CI: 0.41-0.99) (22). This association was differentiated by ambient ozone level (interaction P=0.003). Children having at least one "short" allele of *HMOX-1* and residing in the low ozone communities had 56% lower risk of asthma incidence than those having no "short" allele of *HMOX-1* and living in the low O_3 communities (HR, 0.44; 95% CI: 0.23-0.83). No significant association between *HMOX-1* and asthma risk was found among Hispanics, suggesting differential asthma risk of this genetic variant by race/ethnicity.

TNF mediates asthma occurrence by initiating airway inflammation and generating airway hyperreactivity (62-64). We previously found DNA sequence variant in rs1800629 modified the association between secondhand smoking and risk of respiratory illness-related school absences (65). In the following work, we found more direct associations between *TNF* variant and respiratory illness (*Table S1*). Among children of age 8-11 years old, rs1800629 G/G genotype was associated with 20-30% reduced risk of lifetime asthma (OR, 0.8; 95% CI: 0.7-0.9), life-time (OR, 0.8; 95% CI: 0.7-0.9) and current wheezing (OR, 0.7; 95% CI: 0.6-0.9), and medication for wheezing (OR, 0.7; 95% CI: 0.5-0.8) compared to G/A or A/A genotypes after adjusting for age, sex, race/ethnicity, town, lifetime residence, grade, secondhand tobacco smoke, and *in utero* maternal smoking (66). The protective effects of the G/G genotype on ever wheezing, current wheezing and medication use for wheeze were two times larger in magnitude for children who lived in low ozone (annual average <50 ppb) communities compared to others who lived in high ozone (annual average ≥50 ppb) communities (all interaction P<0.04). No significant interaction was found for rs1800629 with ozone for the association with asthma prevalence. The difference in the rs1800629 G/G genotype effect between low and high ozone exposure was stronger in the *GSTM1* null compared with the *GSTM1* present group. Similarly, the difference in the protective effect of

rs1800629 G/G genotype between low and high ozone exposure was larger among children with *GSTP1* (rs1695) A/A genotype than children with rs1695 A/G or G/G genotypes. The interaction between the rs1800629 G/G genotype and O_3 was also found in the association with bronchitic symptoms among asthmatic children (29). The rs1800629 G/G genotype was associated with 47% reduced risk of bronchitic symptoms for asthmatic children who were exposed to low ambient O_3 after adjusting for age, sex, ethnicity, grade, secondhand tobacco smoke, lifetime residence, and community (OR, 0.53; 95% CI: 0.31-0.91). The protective effect was not found among children living in high O_3 communities.

Arginases play an important role in asthma pathogenesis through nitrosative stress-mediated airway inflammation (64,67-69). CHS results showed both *ARG1* and *ARG2* were globally associated with asthma prevalence (28) (*Table S1*). Compared to the most common *ARG1* haplotype that carried the wild-type allele for seven tagged SNPs, one ARG1 haplotype carrying the variant allele (T) for rs2749935 was associated with a 45% reduced risk of asthma after adjusting for age, sex, ethnicity, child's atopic status, parental history of asthma, parental education, secondhand tobacco smoke, *in utero* maternal smoking, health insurance, and community (OR, 0.55; 95% CI: 0.36-0.84). Each variant allele (G) of *ARG2* SNP rs3742879 was associated with a 31% increase in asthma risk (OR, 1.35; 95% CI: 1.04-1.76). Atopy and ambient O_3 modified the association between one *ARG1* haplotype and the risk of asthma (interaction P=0.04 and 0.02, respectively). This particular *ARG1* haplotype was associated with reduced asthma risk among atopic children or children living in high O_3 communities, but was not associated among non-atopic children or children living in communities with low level of O_3. No significant interactions were found for *ARG2* haplotypes or SNPs with atopy and O_3 in the association with asthma risk. In addition to the observed associations for genetic variations of *ARG*, epigenetic variations in *ARG* were also investigated for its role in modulating Fe_{NO} levels in children. In the CHS, DNA methylation in *ARG2* was significantly associated with airway inflammation among children with mean age of 9 years old (70). A 1% increase in average DNA methylation of *ARG2* was associated with a 2.3% (95% CI: −4.0% to −0.6%) decrease in Fe_{NO} after adjusting for age, sex, race, plate, town, month of DNA collection, asthma medication use, and parental education. This association was more striking among asthmatic children than children without asthma (interaction P=0.01). A similar

interaction was also found for *ARG1*, though little association existed between DNA methylation of *ARG1* and Fe_{NO}.

Another gene involved in the nitrosative stress is *NOS2*, which produces NO in response to environmental stimuli (71-74). CHS results showed seven SNPs in the promoter region of *NOS2* were globally associated with an increased risk of new-onset asthma (P=0.002) and a lower growth of FEV_1 (P=0.02) (75) (*Table S1*). Further analysis indicated that a pair of "yin-yang" haplotypes of these seven SNPs contributed to the association. One copy of the "yin" haplotype (h0111101) was associated with a 49% increased risk of new-onset asthma compared with children without this haplotype controlling for communities with age- and sex-specific baseline hazard (HR, 1.49; 95% CI: 1.03-2.14), and this association was dose-dependent. In contrast, the "yang" (h1000010) haplotype was associated with 34% (HR, 0.66; 95% CI: 0.49-0.88) reduced risk of new-onset asthma and 48.9 mL (95% CI: 11.6-86.2 mL) higher 8-year FEV_1 growth. Interestingly, the increased risk of new-onset asthma for the "yin" haplotype was only found among children who had *GSTM1* null genotype (interaction P=0.002). However, the protective effect of the "yang" haplotype did not vary by the *GSTM1* genotype. To investigate *NOS2* associations with airway inflammations, we found $PM_{2.5}$, DNA methylation in iNOS were jointly associated with Fe_{NO} after adjusting for age, sex, ethnicity, asthma, respiratory allergy, parental education, community, month of Fe_{NO} collection, *NOS2* promoter haplotypes and experimental plate (76). Among children at the highest 10th percentile of iNOS methylation (>56.6%), higher ambient $PM_{2.5}$ was associated with higher Fe_{NO} (P=0.0002); whereas such an association was not significant among children at lower methylation levels.

Because TGF-β1 is involved in airway inflammation (77,78) and remodeling (79,80), the functional polymorphisms in the *TGFB1* gene may play a role in asthma occurrence. We found children with the SNP rs4803457 T/T genotype had a 1.8-fold increased risk of early persistent asthma (asthma as diagnosis before age 3 years with at least one episode of wheeze or asthma medication use after starting first grade) compared to children with C/C or C/T genotypes after adjusting for age, sex, ethnicity, atopic status, parental history of asthma, family income, parental education, *in utero* maternal smoking, number of smokers at home, insurance, and community (OR, 1.81; 95% CI: 1.11-2.95) (81) (*Table S1*). This association was varied by the residential proximity to a freeway (interaction P=0.02). The T/T genotype was associated with more than 3-fold increased risk of lifetime asthma among children living within 500 m of a freeway. However, such an association was not significant among children who lived more than 500 m from a freeway. *In utero* exposure to maternal smoking was previously found to be associated with higher risk of asthma (82). We additionally found such an association can vary by *TGFB1* genotypes (interaction P=0.1) (81). The association between *in utero* exposure to maternal smoking and increased risk of early persistent asthma was only observed among children with T/T genotype (OR, 3.15; 95% CI: 0.81-12.26), but not among children with C/C or C/T genotypes (OR, 0.97; 95% CI: 0.57-1.66).

Discussion

Although air pollution levels have decreased over the last decades (*Figure 1*), the CHS found both regional and traffic-related pollutants are associated with increased asthma prevalence and new-onset asthma, increased risk of both chronic and acute respiratory symptoms for children with asthma, slower lung function development, and higher airway inflammation. Effects of traffic-related pollutions are independent of effects of regional pollutions. The mechanisms underlying the observed associations may involve multiple genetic influences, gene-environmental interactions, and the interactions between air pollution and other exposures such as *in utero* maternal smoking and parental stress.

The CHS results provide evidence that air pollution is a major challenge to public health with respect to childhood respiratory illnesses, especially for countries whose air quality is worse than in the United States. Substantial lifelong adverse effects are a real threat if children's exposures are not reduced. Many approaches can be applied to reduce air pollution exposures including both primary and secondary strategies (30).

"Primary strategies" which reduce the release of air pollutants are critical for the reduction of regional ambient air pollution levels and local traffic-related pollutant levels. Such strategies require the stringent control of automobile and truck emissions. Even under current regulatory levels of air pollutants, adverse effects of air pollution occur for many respiratory illnesses including asthma, low lung function growth, and airway inflammation. These results suggest stricter regulatory standards are needed to prevent adverse health outcomes in the US, Europe and other developed nations. Additional pollutants which are not
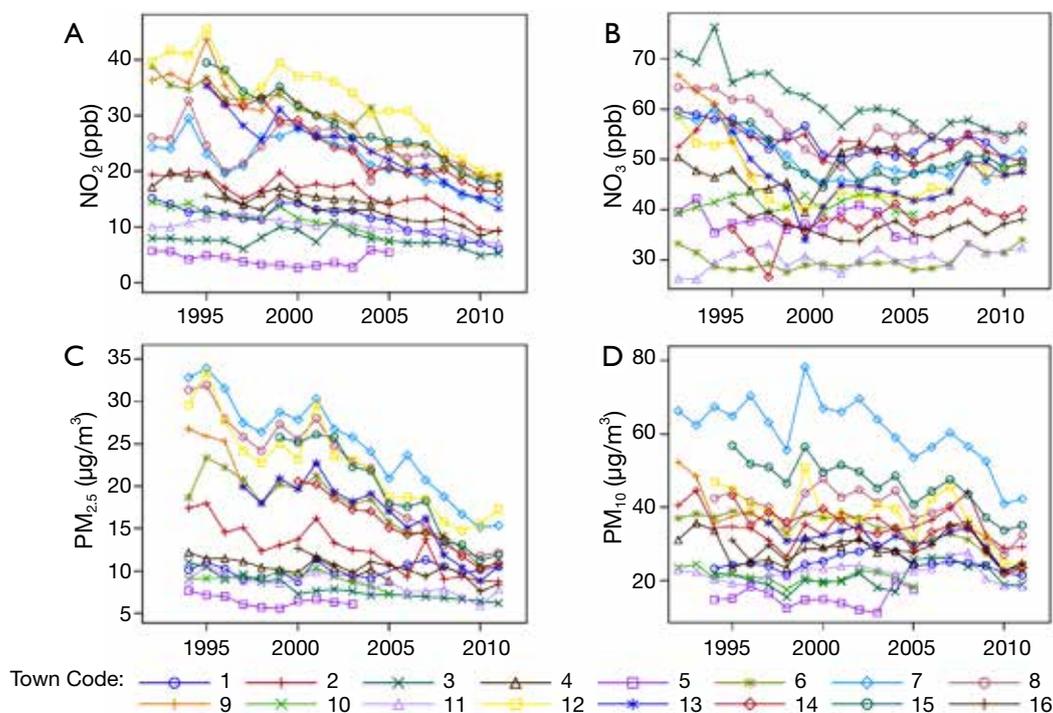
Figure 1 Regional air pollution trends from year 1992 to year 2010 in 16 Southern California communities from the Children's Health Study. Four air pollutants levels are presented: (A) NO_2; (B) O_3; (C) $PM_{2.5}$; and (D) PM_{10}. Town names represented by the town code: 1, Alpine; 2, Lake Elsinore; 3, Lake Gregory; 4, Lancaster; 5, Lompoc; 6, Long Beach; 7, Mira Loma; 8, Riverside; 9, San Dimas; 10, Atascadero; 11, Santa Maria; 12, Upland; 13, Glendora; 14, Anaheim; 15, San Bernardino; 16, Santa Barbara.

included in the current standard need to be targeted in the future based on the growing knowledge of their detrimental impact on public health, including ultrafine particles and PAHs. Traffic-related pollutions are major risk factors, but there are no federal regulatory standards for traffic-related pollutions as for regional air pollutions by NAAQS that was put in place as mandated by the 1970s Clean Air Act. Regulation of traffic-related pollutants would be appropriate to protect children's respiratory health.

Given the limitations in the current regulations and the long time necessary to revise regulations, "secondary strategies" to reduce exposure or to decrease personal susceptibility may also be required. Such strategies could include citing schools and parks away from roads with high traffic volumes; issuing warnings to the public with recommendations for reducing outdoor activity on high pollution days; and minimizing commuting time on roads especially for school commutes.

The strengths of CHS are the long-term, prospective follow-up of five large cohorts of children, with exposure and outcome data collected consistently. We have used

central air monitors to measure regional pollution, and different traffic metrics to estimate traffic-related pollutions. Target genes, GWAS, and DNA methylation data are available for assessing the genetic and epigenetic associations with respiratory health outcomes.

However, we acknowledge that some challenges exist for our future studies. First, although different air pollution exposure models such as dispersion model and land use regression models, and air monitors have been used to estimate and measure ambient and traffic-related pollutions, incorporating activity patterns in time and space (home, school, commute, and workplace) (11) in estimating risk estimate remains a challenge particularly for investigating chronic health effects where personal monitoring (especially in children) is infeasible. Second, identification of the factors involved in asthma etiology has remained a big challenge because of complex interplay between environmental and genetic factors. While candidate gene approach has showed promising interactive effects of ambient air and traffic-related pollution on respiratory health, GWAS efforts has not yielded new susceptibility loci for air pollution mediated

effects. Additionally, much of the variability of asthma cannot be explained by known asthma-related SNPs. Third, use of epigenetics as a mediating factor of ambient air and traffic-related pollution with health outcomes has received interest in scientific community, but there are challenges ahead with evaluating pollution effects in biological samples with mixed cell populations from surrogate tissues (rather than the tissue of interest, lung or airway in this instance, which is infeasible in children or in population-based study), and that epigenetic variation occurring with short-term exposure making it difficult to use these variations for long-term effects.

In conclusion, air pollution has important adverse effects on respiratory illnesses, which may be mediated in part by genes, tobacco smoke exposures and parental stress. Future research is warranted to better define the long-term effects of air pollution including the relationship between early life exposure to air pollution and health outcomes after into adulthood. Individual interventions based on personal susceptibility may be needed to efficiently prevent adverse effects attributable to air pollution while control measures are being implemented. Lastly, more aggressive air pollution regulations are needed to achieve improved public health benefits for future generations of children.

Acknowledgements

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What can individuals do to reduce personal health risks from air pollution?

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Abstract: In many areas of the world, concentrations of ambient air pollutants exceed levels associated with increased risk of acute and chronic health problems. While effective policies to reduce emissions at their sources are clearly preferable, some evidence supports the effectiveness of individual actions to reduce exposure and health risks. Personal exposure to ambient air pollution can be reduced on high air pollution days by staying indoors, reducing outdoor air infiltration to indoors, cleaning indoor air with air filters, and limiting physical exertion, especially outdoors and near air pollution sources. Limited evidence suggests that the use of respirators may be effective in some circumstances. Awareness of air pollution levels is facilitated by a growing number of public air quality alert systems. Avoiding exposure to air pollutants is especially important for susceptible individuals with chronic cardiovascular or pulmonary disease, children, and the elderly. Research on mechanisms underlying the adverse health effects of air pollution have suggested potential pharmaceutical or chemopreventive interventions, such as antioxidant or antithrombotic agents, but in the absence of data on health outcomes, no sound recommendations can be made for primary prevention. Health care providers and their patients should carefully consider individual circumstances related to outdoor and indoor air pollutant exposure levels and susceptibility to those air pollutants when deciding on a course of action to reduce personal exposure and health risks from ambient air pollutants. Careful consideration is especially warranted when interventions may have unintended negative consequences, such as when efforts to avoid exposure to air pollutants lead to reduced physical activity or when there is evidence that dietary supplements, such as antioxidants, have potential adverse health effects. These potential complications of partially effective personal interventions to reduce exposure or risk highlight the primary importance of reducing emissions of air pollutants at their sources.

Keywords: Air pollution; prevention; cardiovascular disease; pulmonary disease; behavior

Introduction

Air pollution is a serious global public health problem that is managed most effectively by collective (societal) action to control emissions of both primary air pollutants and precursors that react to form secondary air pollutants. Unfortunately, in many areas of the world, concentrations of ambient air pollutants currently exceed levels believed to substantially increase risks of acute and chronic adverse human health effects. Affected areas include many of the urban communities where a majority of the world's population now lives and works (1). While waiting for governments to act, or controls to be implemented, are there personal actions that can be taken by individuals to effectively reduce the risks of adverse health effects from air pollution?

As reviewed elsewhere in this issue, scientific studies provide strong evidence for a growing number of adverse health effects of exposure to air pollutants. Given the evidence of harm, the benefits of acting to reduce personal

exposure to air pollution may seem self-evident. Indeed, studies have shown that reductions in exposure at the population level, either due to natural experiments or long-term trends, improve health outcomes (2,3). However, personal-level interventions may have varying degrees of effectiveness for reducing exposure and/or reducing risk, and there has been a dearth of research on actual health outcomes after personal interventions. This is due, at least in part, to difficulties in evaluating the effects of personal interventions on air pollution-attributable health events, which, despite their public health significance, have relatively low frequencies across broad populations. Also, personal actions to reduce exposure to air pollution are best viewed in the context of total risk, because such actions have the potential to cause unintended health effects by altering other risk factors. Interventions aimed at reducing individual susceptibility, or increasing resilience, which may be complementary to actions to reduce exposure, are promising but as yet unproven approaches to reducing risk.

Here, we review and evaluate various individual-level strategies for reducing risk, based on the available evidence to date. The scope of this review is limited to ambient (outdoor-source) air pollution, including exposure to outdoor-source air pollution that occurs indoors, where many individuals spend the majority of their time. The association of indoor and outdoor air pollution is governed by mass balance equations (4), which are modified by many of the interventions to reduce indoor exposure to air pollutants that are reviewed below. Our goal is not to systematically review alternative approaches to reducing exposure and risk from outdoor-source air pollutants, but rather to provide a broad perspective on what we know and what we don't know about individual-level interventions to mitigate health risks from air pollution.

Reducing personal exposure to ambient air pollution

Staying indoors

Personal exposure to ambient air pollutants occurs in both indoor and outdoor environments, and the levels of exposure depend on the fractions of time an individual spends in various indoor and outdoor environments, as well as the concentrations of outdoor-source air pollutants in those indoor and outdoor environments. In the developed world, people spend about 90% of their daily time indoors on average, with about 70% of their daily time in residential

homes (5). There is a lack of information on personal activity patterns in the developing world. Although ambient air pollutants such as particulate matter, ozone, and other gases infiltrate indoors from outdoors, concentrations are generally lower indoors compared to outdoors, and spending time indoors generally reduces exposure to ambient air pollutants. Indeed, environmental protection agencies in a number of countries advise members of the public to remain indoors as part of guidance to reduce exposure and thus acute health risk on high air pollution days (6). However, it is worth noting that infiltration rates vary widely due to differences in building structures, indoor surface materials, air handling systems, building operating conditions, and ambient environmental conditions (e.g., wind speed and direction, temperature, and air pollutant constituents). Concentrations of indoor air pollutants of ambient origin are primarily determined by the process of outdoor-to-indoor transport, which is a function of air exchange rate (building ventilation). Closed windows, usually associated with use of air conditioning in the developed world, can reduce air exchange rates by about 50% (7), leading to reduced infiltration of ambient air pollutants to the indoor environment.

Personal exposure to ambient pollutants in the indoor environment is complicated by indoor air chemistry, through which some ambient pollutants are degraded (e.g., O_3 and nitrate particles) and other new air pollutants are formed (e.g., aldehydes and ammonia) (8). Concentrations of ozone indoors have been found to range widely from 10% to 80% of outdoor concentrations, with means of 40-50%, due to loss of ozone by chemical reactions that occur primarily on interior surfaces (9). The effectiveness of staying indoors to reduce exposure to outdoor-source PM is more limited due to typical penetration factors which can approach unity in the absence of air conditioning (10), and relatively little loss of particles to surface deposition. Evidence that closing windows reduces penetration of PM and associated cardiovascular health risk came from a recent study of 300 healthy adults in Taipei who alternately opened and closed windows at home for 2-week periods. Lin *et al.* [2013] found associations between PM levels and adverse changes in markers of cardiovascular disease risk (increased plasma CRP and fibrinogen, and decreased heart rate variability) after periods with windows open, but no changes with windows closed (11).

Recommendations to spend more time indoors or make buildings "tighter" to reduce penetration of ambient pollutants are further complicated by variable indoor
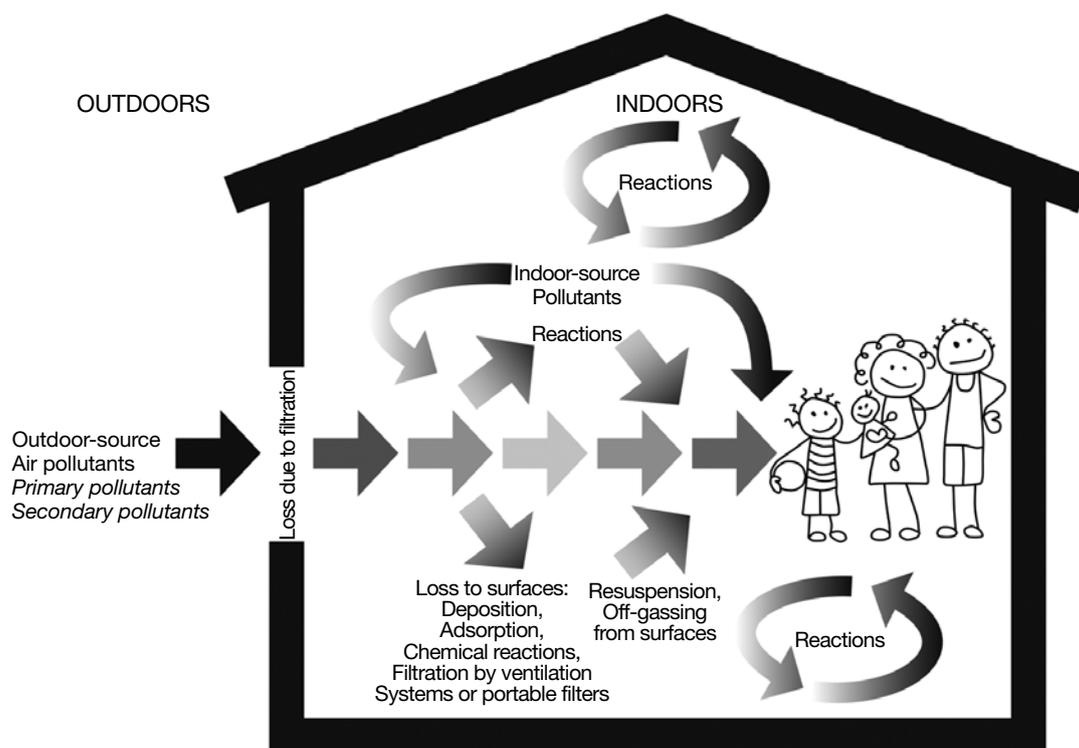
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Figure 1 Schematic diagram illustrating the complex processes that determine exposure to air pollutants of outdoor and indoor origin, including infiltration of outdoor-source pollutants, generation of indoor-source pollutants, chemical reactions in the air and on interior surfaces, adsorption and deposition on surfaces, and re-suspension and off-gassing from surfaces.

sources of air pollutants and the theoretical net risk from the different air pollutants that may be encountered indoors from both indoor and outdoor sources (*Figure 1*). Staying indoors and decreasing home ventilation reduces personal exposures to pollutants of outdoor origin, but at the same time may potentially increase personal exposures and health risks from a variety of indoor-generated primary and secondary air pollutants, including volatile organic compounds from consumer products and building materials, and nitrogen oxides, carbon monoxide and particulate matter from indoor combustion activities such as cooking, wood burning, and smoking tobacco products. For example, Huang *et al.* [2014] reported that levels of indoor PM were associated with decreased heart rate variability (HRV) among housewives. After adjustment for confounders, an interquartile range increase in $PM_{2.5}$ was associated with statistically significant 1.25-4.31% decreases in standard deviation of normal to normal (SDNN) and 0.12-3.71% decreases in root mean squared of successive differences (rMSSD) HRV, and these effects were stronger during stir-frying, cleaning with detergent, and burning incense (12).

Cleaning indoor air

Portable or central air cleaning systems can reduce concentrations of indoor air pollutants, of either outdoor or indoor origin. MacIntosh *et al.* [2008] conducted an indoor air quality study to characterize particle removal efficiencies of several types of central, in-duct air filters/cleaners (13). The authors observed that indoor particles with diameters 0.3-0.5 µm were effectively removed by either placing a 5-inch pleated media filter (model BAYFTAH26M, Trane Residential Systems) or an electrostatic air cleaner in the ventilation duct. The application of the 5-inch pleated media filter reduced the indoor/outdoor (I/O) ratio of 0.3-0.5 µm particles 0.8 to 0.2 (75% decrease, 95% CI: 74-76%), and the electrostatic air cleaner reduced the I/O ratio from 0.8 to 0.05 (a 94% decrease, 95% CI: 93-95%) under typical indoor settings specified in Meng *et al.*, [2009] (7). Macintosh *et al.* [2008] further observed that $PM_{2.5}$ can also be removed effectively by 1-inch and 5-inch pleated media filters (model BAYFTAH26M, Trane Residential Systems) in the ventilation duct (13). Under typical indoor settings,

the 1-inch and 5-inch pleated media filters reduced I/O ratio of $PM_{2.5}$ from 0.40 to 0.27 (a 32.5% decrease, 95% CI: 29-36%) and from 0.40 to 0.08 (an 80% decrease, 95% CI: 79-81%), respectively (7). Practical considerations that may limit the use of increased filtration include added energy costs, noise, and wear and tear to the ventilation system.

Macintosh *et al.* [2010] modeled the health benefits of using a whole house in-duct air cleaner (14). The indoor-outdoor ratio of $PM_{2.5}$ will decrease from 0.57 with natural ventilation (passive air exchange through windows and other openings), to 0.35 with conventional in-duct filtration, to 0.1 with HEPA (high efficiency particle air) in-duct filtration. Based on modeling of the metropolitan areas of Cincinnati, Cleveland, and Columbus, Ohio, reduction in $PM_{2.5}$ I/O ratio from 0.57 to 0.1 after adoption of in-duct HEPA filtration would lead to estimated annual decreases of 700 (0.014%) premature deaths, 940 (0.019%) hospital and ER visit, and 130,000 (2.6%) asthma attacks

In addition to filtration in heating ventilation and air conditioning (HVAC) systems, portable filter-based air cleaners have also been used to reduce indoor levels of $PM_{2.5}$ and assess potential impacts of these reductions on acute health-related biomarkers in controlled experiments. Macintosh *et al.* [2008] reported that the $PM_{2.5}$ can also be effectively removed with a single portable air cleaner with HEPA filter (13). Under typical conditions (7), the operation of a single portable air cleaner with HEPA filter led to a decrease of I/O ratio from 0.4 to 0.14 (a 65% decrease, 95% CI: 63-67%). The actual removal rate is expected to be dependent upon the size of interior space, the ventilation rate, and the flow rate of the portable air cleaner. Bräuner *et al.* [2008] conducted a randomized double-blind, crossover study to quantify the impact of a portable HEPA filter-based indoor air intervention on microvascular function for healthy elderly individuals in Copenhagen (15). The HEPA filter intervention reduced both indoor $PM_{2.5}$ mass concentrations (from 12.6 to 4.7 µg/m³) and particle number concentrations (from 10,016 to 3,206 particles/cm³), leading to an 8.1% (95% CI: 0.4-26.3% improvement in microvascular function. Another study in an area with prevalent wood smoke (Vancouver, BC area) used a similar HEPA filter intervention and reported similar declines in indoor PM levels as well as improved microvascular function (16).

Reducing the effective inhaled dose of air pollution

In addition to staying indoors, with or without further efforts to reduce indoor pollutant levels, reducing exertion can reduce the amount (dose) of air pollutants that are inhaled (17), and can modify the fraction of pollutant deposited or absorbed in different regions of the respiratory tract. For example, an experimental study of healthy adults showed that total respiratory tract deposition of ultrafine particles (diameter <100 nm) was about 5-fold greater during moderate exercise than at rest (18). Compared to the mouth, the nose is a more effective filter for preventing particles and water-soluble gases and vapors from reaching the lung (19). Thus, breathing through the mouth at higher levels of exertion further increases the dose of pollutants that reach the lower respiratory. Another study showed that children 6-10 years old had less nasal deposition of fine particles during light exercise compared to adults, suggesting that limiting exertion in children may be especially important for reducing their exposure to PM (20).

Public health messages in different locales usually refer to avoiding vigorous, extended outdoor activity during air pollution episodes (21). Trade-offs between the health benefits of reduced inhalation exposure to air pollutants and the health benefits of physical activity per se need to be factored into individual recommendations and choices for reducing exercise at certain locations or times in order to mitigate health risks from reduce exposure to air pollution. Physical inactivity is a major risk factor for mortality and morbidity from cardiopulmonary and other diseases, and exercise has been shown to have powerful protective effects for a number chronic disease states (22). European risk assessments showed that, on average, the cardiovascular disease benefits of exercise outweigh the cardiovascular disease risks of increased exposure to air pollution associated with commuting by bicycle alongside urban roadways (23,24). Reviewing available studies that were mostly European, Hartog *et al.* found that, although average levels of exposure to particulate matter were higher during car driving than bicycle riding within the same study, inhaled dose was estimated to be higher during cycling due to increased minute ventilation (23). They conducted a risk assessment based on estimated street-level pollution levels in Amsterdam (in-vehicle and roadway $PM_{2.5}$ of about 35-40 µg/m³), and found that the cardiovascular benefits of replacing short car trips with cycling greatly outweighed the risk from increased exposure to outdoor air pollution. Similar assessments have not been done to compare exercising indoors to exercising outdoors, or avoiding exercise both indoors and outdoors, either regularly or on high pollution days. Comprehensive evaluations would take into account differential individual risk and benefit profiles

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based on susceptibility to adverse effects of air pollution, relative benefits from exercise, dose of air pollution, intensity of exercise, and other factors.

Avoiding outdoor activity when and where air pollutant levels are higher

Ambient air pollution levels vary seasonally, day-to-day, and by time-of-day. For example, ultraviolet light from the sun activates the chemical reactions that form ozone, generally leading to higher concentrations in late morning through early evening (25). Alternatively, ozone concentrations may peak later in the evening or at night in locations that are downwind of ozone formation (25). Levels of air pollutants also vary in different microenvironments, such as outdoors in variable proximity to sources, at home, at workplaces, in schools, in vehicles, etc. Individuals can know when air pollution levels are likely to be elevated either by sensing poor air quality (odor, irritation, symptoms), having knowledge of conditions that tend to lead to higher air pollutant levels in their area, or via public communications based on measured or predicted levels at air monitoring stations. In order to most effectively adjust behavior to reduce exposure and risk, individuals must be able to anticipate when and where air pollutant levels are likely to be elevated above levels thought to confer increased risk.

Ambient air pollutant concentrations are measured by air pollution monitoring networks in a number of countries around the world. These measurements are combined with mathematical models to forecast air pollutant levels over 24 to 48 hours. Both measured concentrations and predicted levels are disseminated to the public in various ways. At present, there is no accepted consensus standardization of approaches or methods, but in general, most authorities convert increasing concentrations of major air pollutants (ozone, $PM_{2.5}$, PM_{10}, carbon monoxide, nitrogen dioxide, and sulfur dioxide) into severity bands labeled with progressive degrees of risk. For example, the US EPA's Air Quality Index (AQI) includes band ratings of 'good, moderate, unhealthy for sensitive individuals, unhealthy, very unhealthy, and hazardous'. The Common Air Quality Index (CAQI), used on the European Union's Air Quality Now website, labels band ratings as 'very low, low, medium, high, and very high'. The AQI, CAQI and other systems use different air pollutant cut-off values to define bands. Therefore, the severity bands are not directly comparable from country to country, even though the severity terms may be the same, because ratings are generally based on how a pollutant

concentration compares to national or other regulatory pollutant thresholds that vary from country to country. All of these indices simplify complex air quality information into relatively straightforward communications to the public, at the expense, to some degree, of precision and accuracy. For example, most consider each air pollutant separately, and many report a single index value based on the pollutant with the highest index value, ignoring poorly-understood, but likely important, interactive effects between different pollutants. The values of most daily indices correspond to standards for daily (24-hour) or shorter averaging times, however, long-term (annual) average standards for the pollutant may be exceeded even if the shorter-term standards are exceeded only infrequently. Aggregate indices that consider the conjoint effects of a number of monitored air pollutants, over various averaging times, have been proposed but have not been incorporated into (26,27).

It is generally assumed that levels of air pollutants that trigger air quality alerts are below thresholds of human detection by odor, irritation, or specific symptomatic responses. However, levels or air pollutants at central monitors can misrepresent local conditions, especially during transient, local air pollution episodes that humans may sense by odor, irritation, or other responses (28). Some studies have found correlations between perceptions of air quality and monitoring data (29-32), but other studies have not (33,34).

The extent to which individuals in different communities are aware of air quality indices or alerts has varied greatly in surveys and focus groups conducted in the US, Canada, and UK (28). There is little data on the extent to which individuals change behavior to reduce exposure either in response to air quality data or perceptions of exposure. In a study of Portland and Houston in the US in 2005-2006, a third of 1,962 participants were aware of air quality alerts, but only 10-15% of individuals reported changing behavior in response to predicted poor air quality, and cited perceptions of poor air quality as driving their behavior, not official advisories (34). Similarly, in a cross-sectional study of 33,888 adult participants, in six states, in the 2005 Behavioral Risk Factor Surveillance System (BRFSS), about a third of adults with asthma and 16% without asthma reported change in outdoor activity due to media alerts (35). Individual perception of poor air quality and health professional advice greatly increased the prevalence of reported behavior change. We could find no studies that have assessed associations between health outcomes

and exposure to public health advisories, physician recommendations, or actual personal behavior change to reduce exposure to air pollutants.

Reducing exposure in microenvironments near sources such as traffic

Air pollutant levels in specific microenvironments are highly variable, and direct measurements or estimates of these levels are rarely available to aid individuals in making decisions about reducing exposure, but some generalizations about expected relative levels of air pollutants under different types of conditions in particular types of microenvironments can be useful. For example, traffic-related air pollution, may present increased risk of adverse health effects to broad populations in many urban areas of the world. Traffic-related pollutants consist of particles and gases emitted from internal combustion engines, their reaction products, tire and vehicle wear, and resuspended road dust. Concentrations of these pollutants decline in steep gradients with distance from roadways, but large urban populations living and/or working in proximity to roadways, as well as commuters on roadways, are among those most likely to be exposed (36). Traffic-related air pollutants have become relatively more important in areas of the world where increased industrial air pollution controls have reduced the contribution of stationary sources to total air pollution emissions. Although per-vehicle emissions have been drastically reduced in many parts of the world, and a recent decline in total vehicle miles travelled (VMT) in the developed world, there has been a rapid increase in motor vehicle ownership and VMT travelled in developing countries (37).

Individuals can reduce exposure to air pollutants and potential adverse health effects by avoiding regular physical activity alongside high-traffic roadways or near other sources of combustion such as burning of wood, biomass, or other materials. Exposure to traffic pollutants can be a rational consideration in choosing walking, biking, or exercise routes. In general, traffic pollution concentrations fall rapidly at distances from roadways, approaching background within about 500 meters, assuming no other local sources are nearby (36). Various web-based applications can assist individuals in finding alternative routes (e.g., http://www.cyclevancouver.ubc.ca/cv.aspx in Vancouver, Canada).

Individuals who commute to work in personal vehicles or public transportation receive a substantial portion of their daily dose of air pollution during commuting activities (38,39). Pollutants emitted by nearby vehicles are the main source of on-roadway exposure. Most air intake filters in passenger vehicles are relatively low efficiency and air pollutants enter through open windows, leaks in door and window seals, and other openings. Vehicle operating conditions have been shown to strongly influence concentrations of air pollutants in vehicles, with I/O ratios ranging from close to 1.0 with windows open to 0.2 or less with windows closed and ventilation set to recirculate cabin air. Vehicle speed and age also strongly affecting I/O ratios (40). Reductions in I/O ratio are generally greater in vehicles with cabin recirculation filters that are becoming more common in later model passenger vehicles. Reductions of in-cabin PM exposure of up to 40% with cabin filters have been observed (41). Among a panel of 60 healthy adults commuting 2 hours by car in Taipei, Chuang *et al.* [2013] found that associations between in–vehicle $PM_{2.5}$ and acute decreases in HRV were modified by keeping the ventilation system in recirculation mode with the air conditioner on (42). An interquartile range increase in $PM_{2.5}$ was associated with a 4.8% (95% CI: 2.9-6.7%) decrease in SDNN and 6.9% (95% CI: 5.9-7.9%) decrease in RMSSD with air conditioner off, compared to 0.7% (95% CI: 0.3-1.1%) decrease in SDNN and 0.1 (95% CI: −1.4-1.6) decrease in RMSSD with air conditioner on, with P value for the interaction in both comparisons <0.01.

Personal protective equipment—respirators

In some urban areas around the world, it is not unusual to observe individuals wearing various types of respirators on urban streets in order to reduce exposure to air pollutants. The ability of a respirator to remove contaminants from inhaled air depends on the contaminant, type of filter or adsorbent material, respirator type and conditions of use. Although, relatively inexpensive respirators with filter material for particulate matter are widely available, no single absorbent, or available combination of adsorbents, can efficiently remove the various gas phase air pollutants that may be encountered. Gaseous pollutants can be removed based on their physicochemical properties, such as reactivity, molecular weight, and volatility. Therefore, the removal mechanism for different gaseous pollutant can be quite different, i.e., chemical reaction *vs.* adsorption; and a particular adsorbent is only suitable for removal one or a groups of pollutants with similar physicochemical properties. In general, assuming that the filter or adsorbent material

is appropriate for the type of air pollutant, the efficiency of air pollutant removal by tight-fitting negative pressure respirators depends largely on the quality of the individual's face seal. With a proper seal, the National Institutes for Occupational Safety and Health (NIOSH), which certifies respirators in the US, assigns a "protection factor" of 10 to the filtering-facepiece respirators (commonly referred to as a facemask) (43). This means that when properly worn by an individual who has been fit-tested, these respirators are expected to reduce the concentration of the air contaminant inside the facepiece to ≤10% of the concentration outside the facepiece. Fit testing and instruction in proper selection and use of respirators is a part of standards and practices for industrial respiratory protection in many countries, but may be unavailable, and perhaps impractical, when respirators are used by large populations in non-industrial settings.

Limited evidence suggests that the use of negative pressure air-purifying respirators under experimental conditions may reduce cardiovascular risks from exposure to urban PM. Langrish *et al.* [2009] and Langrish *et al.* [2012] conducted controlled intervention studies with healthy individuals and patients with coronary heart disease, who walked along an assigned route in the center of Beijing for two hours with and without a negative pressure air-purifying respirator (44,45). Among 15 healthy subjects, the authors reported that wearing the facemask was associated with decreased systolic blood pressure during the walk compared to not wearing the facemask (121 mmHg without mask *vs.* 114 mmHg with mask, P<0.01), and increased heart rate variability (SDNN 61.2 ms without mask *vs.* 65.6 ms with facemask, P<0.05) over 24 hours, both indicators of decreased cardiovascular risk (44). Among 98 patients with heart disease, similar effects were observed, with the addition of reduced ST-segment depression (−142 *vs.* −156 μV, P=0.046) over 24 hours comparing walks with the facemask to walks without the facemask (45). This is encouraging, because presumably beneficial cardiovascular effects were nonetheless observed despite the added work of breathing imposed on the wearer by this type of negative-pressure, air-purifying respirator.

However, wearing this type of respirator has physiological effects that may confound cardiovascular effects that might be attributed to reductions in exposure to PM. For example, a study of healthy men wearing negative-pressure, air-purifying respirators while exercising at various levels on a treadmill found monotonic increases in heart rate progressing from rest to increasing levels of exercise, but systolic BP showed a biphasic response,

being significantly lower at rest and higher at high levels of exercise (46). Thus, net benefit of wearing a respirator, especially in a susceptible individual for whom increased work of breathing is important, may be a complex function that does not translate simply from the actual reduction in particle exposure. Any net benefits of the practice of wearing respirators to reduce risk from ambient particulate matter air pollution will depend on the exposure reduction efficiency of the respirator and the concentration and potency of the particulate matter mixture, as well as any detrimental physiological and/or psychological effects of respiratory use. Results from single studies, like the Langrish *et al.* studies in Beijing, are not easily generalizable to other locales, populations, and circumstances (44,45). Additional studies are needed to replicate these findings and to clarify conditions of use that will optimize outcomes in different groups.

Regardless of the level of effectiveness at reducing exposure to air pollutants, the use of personal respiratory protection may be limited by individual and public acceptability, based on comfort, appearance, and inhibition of communication and other activities. Many find respirator contact with the face, perceived increased work-of-breathing and thermal discomfort intolerable for more than short periods of time. One study found that air temperatures at the face averaged 7.5 deg C higher during use of respirator at rest and during exercise (46). Some individuals may experience anxiety similar to claustrophobia when wearing a respirator, and facial features and facial hair may make it impossible to achieve an acceptably tight fit (43).

Knowing if one is more or less likely to be susceptible

In addition to knowing when and where exposures are, or are likely to be, more intense, individuals can better optimize the balance of personal risks and benefits by knowing if they are more likely than the general population to be particularly sensitive to harmful effects of different air pollutants. While children and young adults may be highly susceptible to some of the subclinical changes caused by air pollution (47,48), clinical events attributable to air pollution, such as myocardial infarction, stroke, or hospitalization for respiratory failure or heart failure, will of course be much more common in older individuals with advanced underlying disease such as COPD or atherosclerotic plaques. Individuals vary in sensitivity to adverse effects of air pollutants, and more-sensitive

individuals are likely to obtain more benefit from efforts to reduce personal exposure (49). Generally, individuals with chronic cardiovascular or respiratory disease, children, fetuses, and the elderly are thought to be most sensitive to the major "criteria" air pollutants. Adverse effects can be distinguished as either chronic disease due to cumulative exposure over time, or acute effects of short-term exposure. For acute effects, individuals with asthma, COPD, diabetes, and underlying atherosclerotic cardiovascular disease are regarded as among the most vulnerable, due to demonstrated risk of exacerbation of these or related conditions with short-term exposure to elevated levels of air pollution (50,51). Emerging evidence suggests that the developing fetus may be especially sensitive to maternal exposure to air pollutants (48,52). In general, children and the elderly are thought to be more susceptible to air pollution effects; children due to increased body-size-adjusted dose, immature detoxifying mechanisms, and developing organ system and the elderly due to increased prevalence of chronic disease or other factors contributing to age-related loss of resilience and increased risk (53). There is some evidence that genetic variants such as polymorphisms in antioxidant genes may confer increased risk from air pollutants [see reviews (54,55) and Chen *et al.* in this issue (56)]. Genetic, as well as epigenetic, variation holds promise for future tailoring of interventions based on individual susceptibility, but at present there are no clinically applicable tests for varying levels of individual sensitivity to the chronic or acute health effects of air pollutants.

Interventions to modify individual susceptibility

Chronic medical conditions, such as asthma, COPD, and traditional cardiovascular disease risk factors may make individuals more susceptible to the adverse health effects of air pollution. Effective medical treatment and management of these conditions seems to be a logical first step for ameliorating increased risk from ambient or indoor pollutants although no epidemiological or clinical studies have provided direct evidence that such treatment modifies the adverse effects of air pollution. Consensus standards for managing asthma, COPD, and heart disease do include limiting exposure to ambient air pollution among guidelines for preventing exacerbation of these conditions (49,57,58). However, there is currently no direct evidence that improved clinical management reduces risk of adverse health effects from exposure to air pollution.

We know from cohort studies (Women's Health Initiative, Six Cities) that chronic exposure to higher (not necessarily high in global terms) levels of air pollutants are indisputably associated with development of COPD and atherosclerotic CVD, including mortality (59-61). From panel studies we know that day to day, and even hour to hour changes in particulate pollution levels substantially increase risk for MI, heart failure, and stroke. From other panel studies, we have learned a great deal about the pathophysiology of these clinical outcomes (47,62-64). These studies have confirmed the important, and to some extent reversible roles for pathophysiologic processes such as oxidative stress, pulmonary and systemic inflammation, vascular/endothelial dysfunction, and increased signs of coagulation, as key processes that wax and wane acutely with air pollution and likely trigger acute events and contribute to development of chronic disease such as ASCVD. Critically, these same processes are also invoked in the pathophysiology of heart and lung disease, independent of air pollution. The overlap is remarkable but not surprising because the disease endpoints are the same. Thus, we must ask what we can learn from preventive pulmonary and cardiology methods that are applicable to the special case of air pollution's effects on heart and lung disease.

Unfortunately, the cupboard is rather bare in terms of proven interventions for the general cardiologic case that can then be applied to the more specific case of air pollution, where no such experiments have been tried. The Mediterranean diet has been shown to both decrease total and cardiovascular mortality and to be associated with improved biomarkers of cardiovascular risk (65). However, multiple randomized controlled tests of antioxidant and vitamin supplements, based on the confirmed high levels of antioxidants in the Mediterranean and other beneficial diets, and confirmed activity in *in vitro* and *in vivo* laboratory tests, have not shown benefit and in some randomized trials have proven harmful (66). Thus antioxidant supplementation cannot be confidently recommended to counteract air pollution. Indirect antioxidants such as sulforaphane (in broccoli sprouts) have shown promising acute pilot effects but are not regarded as proven for populations (67). Statins are antioxidant as well as lipid-lowering, but these are again unproven in a population without a primary lipid-lowering indication (68,69).

Fish oil supplementation has shown beneficial effects not only on blood lipids but also on heart rate variability (68,69). Beets, and other foods rich in nitrates, do demonstrate a beneficial effect on blood pressure (70) but there is no

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outcomes-based evidence that supplementation of dietary nitrates, or pharmacologic control of blood pressure, is protective against cardiopulmonary effects of air pollution.

Aspirin is widely recommended and effective for reducing MI and stoke risk after a primary event and given the data showing over a doubling of MI risk and increased platelet activation with acute exposure to ambient PM, this is an attractive intervention (47,71). However, calculation of risk-benefit and duration of therapy and actual change in health outcomes or biomarkers associated with air pollution are lacking at this time. Thus, despite compelling mechanistic evidence, no specific recommendations for dietary changes or chemoprevention can be made beyond those already made for prevention of heart and lung disease in general.

Conclusions

Limited evidence supports individual actions to reduce cardiopulmonary health risks from personal exposure to ambient air pollutants by staying indoors and limiting physical exertion when air pollutant levels exceed health-based thresholds. Improved management of chronic diseases that are affected by air pollution will decrease overall risk of adverse outcomes. Available evidence is less clear about the benefits of efforts to reduce susceptibility to air pollution by pharmaceutical or chemopreventive approaches. It is clear that the relative contribution of indoor- and outdoor-generated pollutants to personal exposures depends on multiple factors, including the type of pollutants, building structure, indoor sources, and personal activities (7). Health care providers and their patients should consider these factors and tailor interventions to individual circumstances in order to maximize the net exposure reduction based on individual circumstances (53). While it may not be practical to explicitly and reliably quantify these exposures, if indoor pollutant generation can be minimized, then staying indoors makes more sense. In addition to the balance of air pollutant exposures, benefits of any reduction in exposure to air pollutants must be weighed against the physical and mental health benefits of outdoor activity. In addition to reducing outdoor activity on high pollution days, these public health messages may discourage outdoor activity at other times. The benefits of physical activity may be especially great for individuals who are also more sensitive to air pollution, such as those with heart and respiratory disease. On the other hand, the balance will tip more towards limiting activity as air pollution concentrations reach higher levels, on days with particularly poor air quality, or in areas with chronically elevated levels of air pollution. Encouraging individuals to exercise at locations and times when air pollutant levels are lower may help to preserve the benefits of exercise, while minimizing the health risks from exposure to air pollution. To our knowledge, no explicit formulae for calculating and optimizing this risk-benefit ratio are available at this time.

Acknowledgements

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Mechanistic impact of outdoor air pollution on asthma and allergic diseases

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Abstract: Over the past decades, asthma and allergic diseases, such as allergic rhinitis and eczema, have become increasingly common, but the reason for this increased prevalence is still unclear. It has become apparent that genetic variation alone is not sufficient to account for the observed changes; rather, the changing environment, together with alterations in lifestyle and eating habits, are likely to have driven the increase in prevalence, and in some cases, severity of disease. This is particularly highlighted by recent awareness of, and concern about, the exposure to ubiquitous environmental pollutants, including chemicals with oxidant-generating capacities, and their impact on the human respiratory and immune systems. Indeed, several epidemiological studies have identified a variety of risk factors, including ambient pollutant gases and airborne particles, for the prevalence and the exacerbation of allergic diseases. However, the responsible pollutants remain unclear and the causal relationship has not been established. Recent studies of cellular and animal models have suggested several plausible mechanisms, with the most consistent observation being the direct effects of particle components on the generation of reactive oxygen species (ROS) and the resultant oxidative stress and inflammatory responses. This review attempts to highlight the experimental findings, with particular emphasis on several major mechanistic events initiated by exposure to particulate matters (PMs) in the exposure-disease relationship.

Keywords: Air pollution; asthma; allergic disease; particulate matter (PM); polycyclic aromatic hydrocarbon (PAH); transition metal; aryl hydrocarbon receptor (AhR)

Allergic diseases and asthma still remain a critical public health, medical and economic problem, and are, in fact, among the major causes of illness and disability for all ages, particularly in Taiwan. Unfortunately, the trend of disease incidence is still on the rise and has caused significant economic burden to the general public. Particularly, data from a recent survey of 24,999 first-grade students in Taiwan revealed an alarmingly high prevalence of physician-diagnosed asthma, allergic rhinitis, and atopic eczema, with 13.0%, 33.7% and 29.8%, respectively (1). This is, indeed, a disturbing trend, considering also the fact that

allergic diseases are often a chronic condition for life. The initial assessment of Taiwan's National Health Insurance (NHI) expenditures for asthma* indicated that the annual healthcare cost is estimated to be more than 4 billion (NT$), when all outpatient visits and hospital admissions with asthma as one of the first three diagnoses were included*. In fact, the treatment and care for severe asthma represented more than half of the expenditure incurred. This significant increase in health cost is alarming, which highlights an urgent need for better understanding of the etiology and its causative mechanisms in order to improve treatment and

develop effective prevention strategies.

Mechanistic aspects of air pollution's effects

In the past decades, the increase in our understanding of the pathogenesis of allergic diseases has been substantial, and a Th2-dominant immune response leading to tissue inflammation and remodeling has long been considered as the underlying mechanism (2). Recently, new evidence has also been presented in support of the importance of innate immunity at the level of dendritic cell and epithelial cell response in disease expression (3,4), and additional types of T-cell responses [e.g., Th17 and innate lymphoid cells (iLCs)] also likely contribute to the disease progression (5,6). In the context of asthma, which is characterized by intermittent reversible airway obstruction, persistent pulmonary inflammation, fibrosis and enhanced airway hyperresponsiveness are considered to be the leading cause, particularly in severe cases (7,8). However, our current understanding of the molecular and genetic basis of asthma and allergic diseases is still incomplete; also, a significant portion of the adult asthma population appears to be "non-atopic", but the etiology of this unique population remains to be defined. In this context, there has been a wealth of evidence linking exposure to both outdoor and indoor air pollution, especially airborne PMs, to adverse effects on the allergic diseases, where PM's surface materials are likely to be the culprit and many immune regulatory cell types and pulmonary resident cells could very well be the targets. Notably, local and systemic oxidative stress has emerged as the likely common link between pulmonary exposure and immune regulatory effects, and as a converging point of their mechanistic impact (9,10).

Many reviews have discussed the potential mechanisms of each individual component associated with PMs; for example, the functional impact of air pollutant gases, such as ozone, NO_2 and CO, has been extensively studied and several recent reviews (11,12) have provided a detailed account of their important roles and, thus, will not be the subject of this current review; rather, this review attempts to illustrate a series of mechanistic events as the result of exposure to PMs and their surface chemical and metal components to establish the exposure-disease mechanistic relationship. Specifically, the focus will be on the role of oxidative stress response that may explain the relationship between particulate air pollutants and allergic diseases; also, the recent documented importance of the aryl hydrocarbon receptor (AhR) with high binding affinity to environmental

PAHs in immune regulation is discussed. While PMs are also known to contain biological components, including allergens and those from bacterial sources [e.g., lipopolysaccharide (LPS)], the impacts of those biological materials have been extensively reviewed and will not be the emphasis of this review.

PMs and their modulatory activities

PMs, a major component of air pollution, are generally derived from various anthropogenic, industrial activities and traffic-related sources primarily by coal and oil fuel combustion, while the combustion-derived particles can be originated from a number of sources, including diesel soot, welding fume, carbon black, coal or oil fly ash. In fact, human exposure to those particles has increased markedly over the past century; in particular, diesel exhaust has been estimated to account for up to 80% of human exposure. PMs typically contain a mixture of particles with different origins, size and composition, and are commonly grouped into the following categories: PM_{10} is defined as PM less than 10 mm, while those between 0.1 and 2.5 mm ($PM_{2.5}$) are 'fine' in size and particles less than 0.1 mm are regarded as being 'ultrafine' (UF). It is worth noting that PM_{10} is often referred to those containing both fine and ultrafine particles (UFPs), and $PM_{2.5}$ encompasses both fine and UFPs. World Health Organization (WHO) has set the daily exposure limits at 25 and 50 $\mu g/m^3$, respectively, for $PM_{2.5}$ and PM_{10}, and an annual $PM_{2.5}$ limit at less than 10 $\mu g/m^3$ (13). At present, there are no recommendations made for daily or annual exposure limits for UFPs globally.

The potency of PMs in mediating the health impact is dependent, in part, on their deposition in the airways and the composition of their surface components (14). A variety of PMs are primarily composed of carbon cores and a host of organic chemicals and reactive metals on their surface, the composition of which may vary, depending on the source of the pollutant, the meteorological conditions, industrial activity and traffic density. PM_{10} are usually trapped in the upper airways, while $PM_{2.5}$ particles, particularly the UFP fraction, can penetrate deep into the alveolar portions of the lung and those water-soluble components has been demonstrated, in fact, to be able to translocate directly through the alveolar capillaries into the circulation. Because of this size-dependent deposition, $PM_{2.5}$ and UFPs, particularly diesel exhaust particles (DEPs) and residue oil fly ash (ROFA), have received much attention, since they possess, inherently, larger surface area for a

given mass, and hence carrying larger amounts of reactive chemicals into the peripheral airways.

While the exact mechanisms of PM's health effects remain to be fully defined, recent advancement in the study of various cellular and animal models has clearly documented the role of PMs in regulating many aspects of the respiratory functions, innate and adaptive immunity. In this regard, ROFA with chemically complex mixture of sulfates, carbon- and nitrogen-containing compounds and metals (primarily vanadium), has the capacity to influence immunity and induce injury in various experimental systems. Notably, several early studies have provided evidence that the composition of soluble metals and sulfate leached from ROFA is critical in the development of airway hyper-reactivity and lung injury (15). For example, in respiratory epithelial cells, ROFA mediated its impact on activation of signaling events and cytokine/chemokine production (16). Interestingly, redox-active vanadium, a major ROFA surface metal, was shown to be able to generate similar responses, whereas catalytically active iron and nickel compounds had no such effect. Conversely, treatment of the cells with deferoxamine, a metal chelating agent, reversed ROFA's effects (17). *In vivo*, monkeys exposed to vanadium-containing compounds demonstrated neutrophilic inflammation in both the bronchi and the distal lung, which was accompanied by significant obstruction in the airways, supporting their importance in mediating ROFA's effect (18). In addition, ROFA has been shown to enhance allergen-induced pulmonary allergic response in mouse models of asthma, with significant elevations in allergen-mediated eosinophilia and airway hyperresponsiveness (19-22). Again, this effect could be reproduced by the administration of ROFA's metal leachate or its individual metallic constituents and could be abrogated by the addition of metal chelating agents (22,23). Interestingly, a very recent study (24) described a new mechanism of vanadate's effect through augmenting the function of Toll-like receptor 4 (TLR4) by suppressing TLR4 degradation. These results suggest, therefore, that ROFA's surface metals may be the key determinants in the induction and/or amplification of allergic responses.

Besides ROFA, DEPs are one of the most extensively studied PMs, which generally are within the $PM_{2.5}$ category and account for most airborne PM exposure in major cities (25). Studies have shown that DEPs can modify the immune response and airway inflammatory processes in both animals and humans (26,27). The adjuvant activity of DEP is particularly noted in an elegant study of DEP's effect on the development of IgE response to a "neo-antigen", keyhole limpet hemocyanin (KLH), to which humans are not exposed (28). Diaz-Sanchez and her colleagues presented evidence showing that intranasal exposure of human subjects with KLH alone led to the generation of anti-KLH IgG and IgA, but not IgE, Abs. In contrast, when the study subjects were exposed to DEPs prior to the KLH challenge, the majority of the subjects developed anti-KLH—specific IgE Abs, while the levels of specific IgG and IgA Abs were similar to those with KLH sensitization alone. The elevated IgE levels were found to be associated with increased IL-4, but not IFN-γ, levels in the nasal lavage fluid. These studies demonstrate that DEPs can act as mucosal adjuvant in enhancing the IgE response to allergens and amplifying the allergic responses. Further, the finding of DEP's adjuvant activity was corroborated from the studies of allergen-induced responses, where DEP challenge significantly enhanced nasal ragweed-specific IgE and skewed cytokine production to a Th2 pattern in humans (29). Further, DEP's enhancing effect has recently been broadened to include its prenatal influence on allergic sensitization. Interestingly, the risk was found to be particularly evident in subjects carrying homozygous variant genotype of the gene encoding glutathione-S-transferase M1 (GSTM1) (30). The fact that aeroallergens can also be found to associate with PMs highlights the importance of PM exposure in contributing to the enhanced allergic response (31).

Moreover, it has recently been demonstrated that co-exposure to DEP and HDM significantly enhanced airway hyperresponsiveness as compared with HDM exposure alone, concomitant with a mixed Th2 and Th17 response, including IL-13/IL-17A double-producing T cells (32). Neutralization of IL-17A prevented DEP-induced airway hyperresponsiveness. Significantly, high DEP-exposed children with allergic asthma, 32.2% of them had more frequent asthma symptoms and higher serum IL-17A levels as opposed to low DEP-exposed children, suggesting an expansion of Th17 cells in DEP-exposed subjects with severe asthma. In addition, PM has recently been shown to suppress regulatory T cells (Treg) through its ability in causing hypermethylation of the gene encoding the Treg-transcription factor, Foxp3, in the peripheral blood of children with asthma (33). The reduction in Treg population upon exposure to PMs could be important in potentiating the allergic response, although further studies are needed to confirm this finding. It is also of interest to note that acute exposure of infant mice to a chemically defined environmentally persistent free radicals (EPFR)

with an organic pollutant 1,2-dichlorobenzene (DCB-230) led to epithelial-to-mesenchymal transition (EMT) in the lungs, as evidenced by lineage tracing studies and by the expression of both epithelial E-cadherin and mesenchymal α-smooth muscle actin (α-SMA) proteins (34).

In a direct intervention-type of the study, exposure of healthy human subjects to DEP (300 μg/m^3 for 1 hour) in a chamber caused an increase in inflammatory cells (neutrophils, B lymphocytes, mast cells, CD4$^+$ and CD8$^+$ T lymphocytes) along with up-regulation of ICAM-1 and vascular cell adhesion molecule-1 in the lungs (35). However, when atopic asthmatics were exposed to the same amount of DEP, no significant changes in the levels of neutrophils, eosinophils and IL-8 expression were noted, due, perhaps in part, to the pre-existing inflammatory response (36). Further, exposure to DEP (PM$_{10}$, 300 μg/m^3) enhanced the expression of EGFR and its phosphorylation at the tyrosine residue (Tyr 1173), concomitant with increased activation of the JNK, AP-1, p38 MAPK and NF-kB pathways as well as cytokine production in the airway mucosa of healthy subjects (37,38).

While these results are informative, the mechanisms through which exposure to ROFA or DEP mediates the Th2, Th17, IgE and epithelial responses remain unclear. It is also, at present, unclear as to the exact components responsible for the observed effects mediated by PMs or DEP. In general, DEPs consist of polycyclic aromatic hydrocarbons (PAHs) and transition metals, and as in ROFA, DEP-bound transition metals may play a role in generating oxidative stress and inflammatory responses. Further, their hydrophobic nature allows them to diffuse through cell membranes, and PAHs may interact with a unique cellular chemical sensor, aryl hydrocarbon receptor (AhR; see below), thereby activating the cellular response. In fact, recent discoveries regarding AhR and environmental toxicant interaction and its influence on immune responses have highlighted the potential importance of AhR in linking the environmental exposure and the development of asthma and allergic diseases.

Involvement of AhR signaling pathways in controlling cellular homeostasis

AhR is a ligand-activated transcription factor from the Per-Arnt-Sim (PAS) superfamily and has been shown to be involved in maintaining cellular homeostasis [for recent review, see (39,40)]. Originally discovered as a receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), AhR has

also been recognized as a receptor for many of the common environmental contaminants, including polychlorinated biphenyls (PCBs) and PAHs, such as benzo[α]pyrene (BaP). Upon ligand interaction, AhR in the cytoplasm translocates into the nucleus and binds to the specific regulatory DNA sequences known as dioxin response elements (DREs) located within the promoters of target genes. Those target genes include those involving detoxification enzymes and activities, e.g., CYP1A1 and CYP1B1 etc., as well as those known to be critical in immune regulation, including inflammatory (TNF-α and IL-6) and T-cell differentiation genes (Foxp3, IL-17 and GATA3) (41). Also, beside the direct effect on target gene transcription, AhR may also influence the activation of key signaling events involving NFkB, MAPK, STAT1 and STAT5 that have been observed in many experimental systems (42). In particular, recent discoveries of AhR and its influence on the balance of Tregs and Th17 cells (43,44), and its impact on γδ$^+$ T (45), intraepithelial lymphocytes (46), lymphoid follicles (47), as well as dendritic cell function (48) highlight the potential importance of the AhR-ligand axis in the expression of allergic and inflammatory diseases. We have recently discovered that in stimulated mast cells, a critical cell type in the regulation of allergic responses and mucosal immunity (41), exposure to environmental chemicals, such as dioxin and BaP, resulted in enhanced mast cell signaling, degranulation, mediator and cytokine release, as well as the *in vivo* anaphylactic response (49). Interestingly, known endogenous ligands, including the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ) and a key metabolite of tryptophan, kynurenine, were able to mediate similar effects (49,50). As a corollary, two recent studies (51,52) supported the importance of the AhR ligands in regulating the mast cell response. Therefore, it is logical to speculate that the AhR pathway may serve as a critical bridge linking environmental exposure and allergic diseases. In support of this, experimental evidence has suggested that TCDD, the classical AhR ligand, was able to enhance IgE production and aggravate allergic diseases (53). Also, previous studies have pointed to a possible effect of the exposure to PAHs and allergic diseases (54).

Moreover, the phenotypic alterations found in AhR-null mouse models have suggested the endogenous function of AhR, independent of xenobiotic metabolism, in controlling cellular homeostasis (47,49). This was especially noted in our recent finding of significant mast cell deficiency in AhR-null mice, due to defective calcium signaling and mitochondrial function. Consequently, AhR-null mast cells responded

poorly to stimulation, demonstrating a critical role of AhR signaling in maintaining mast cell homeostasis (49). These results suggest that exposure to environmental pollutants may modify the homeostatic state through their ability to influence the immune response directly or indirectly through the disruption of the normal endogenous function of AhR, and together may contribute to the pathogenesis and symptoms of allergic diseases. In this context, AhR is known to be a chemical sensor initiating the detoxification process through its ability to induce both phase I and II enzymes. Disruption of this critical function by competitive exogenous ligands or by mechanisms causing fluctuation in its expression level would, therefore, have a direct impact on the normal functioning of the detoxification process. It is, perhaps, the dysregulated detoxification, or increased metabolites thereof, in enhancing the cellular response and/or causing the inflammatory responses following the environmental insult. Further investigations into these interesting possibilities may provide additional insights in our understanding of the exposure-disease relationship.

Oxidative stress response and its functional consequences

As discussed above, one of the most consistent PM-mediated immunoregulatory and respiratory effects involves PM-driven oxidative stress (13), a feature likely to be mediated by those PMs referred to as "EPFRs", in which PAHs are chemisorbed to the surface of PM through transition metal oxides. Indeed, the role of oxidative stress in allergic diseases is gaining increasing scientific attention. Exposure to a variety of pollutants has been shown to be able to generate a collection of oxygen-derived free radicals and oxidants, such as hydrogen peroxide and peroxynitrite. For example, combustion-derived PMs are known to be highly oxidizing and are capable of generating free radicals, through, in part, their surface metals involved in redox cycling or the depletion of anti-oxidant glutathione and protein-bound sulfhydryl groups (55,56). It has been postulated that transition metals found in ROFA, particularly vanadium, may generate ROS in an Fenton-like chemical reaction, leading to the subsequent activation of cellular signaling, transcription and induction of inflammatory mediator release and airway hyperresponsiveness [for detailed review see (10) and the references therein].

Oxidative stress occurs not only as a result of environmental exposure to air pollution but also from inflammation, thus perpetuating the oxidative stress response (57,58). Various components of air pollution, including gases and PMs, are known to be able to induce oxidative stress either by direct induction of reactive oxygen species (ROS) or through the secondary induction of local inflammatory processes which lead to the secondary production of ROS (59). Other components of air pollution that may contribute to oxidant generation include transition metals, such as chromium, iron, manganese, vanadium and copper. While the detailed mechanisms remain to be elucidated, oxidative stress has been shown to mediate multiple effects and is associated with activation of inflammatory cells and release of pro-inflammatory cytokines and mediators (60,61). One of the possible earlier events may be as the consequence of the cellular detoxification itself in the endoplasmic reticulum (ER), particularly with regard to the detoxification process of PAH exposure. ER is critical for protein folding and secretion, calcium homeostasis, lipid biosynthesis and detoxification of xenobiotic substances. Any disturbance in the redox balance within the ER may lead to the accumulation of unfolded proteins in the ER lumen, a condition referred to as "ER stress". In response to ER stress, an adaptive signaling pathway, known as the unfolded protein response (UPR), is activated, which intersects with many different inflammatory and stress signaling pathways (62,63).

ER stress has been implicated in many inflammatory diseases (64,65), although the roles of ER stress in the pathogenesis of allergic inflammation remain to be elucidated. A recent study (66) of $PM_{2.5}$ exposure and ER stress response may shed some new lights on this possibility. The study by Laing *et al.* provided evidence that exposure of epithelial cells to $PM_{2.5}$ differentially activated the UPR branches, leading to ER stress-induced apoptosis. Also, we have found that exposure of mast cells to AhR ligands resulted in increased expression of several ER stress response-associated markers (unpublished observation). Moreover, AhR ligands have been shown to increase ROS production, perhaps as the consequence of the increased expression of AhR's target genes, CYP1A1 (49,67) and a member of the membrane NADPH oxidase complex, p40phox (68). Therefore, chronic exposure to $PM_{2.5}$ or its derivatives may interfere with the normal functioning of the ER and stimulate the ER stress response via, in part, AhR, leading to dysregulated allergic response.

Mechanistically, increased oxidative stress is known to mediate the activation of various signal transduction pathways and transcription factors, including MAPK kinases, phosphoinositol-3 kinase, NF-kB and AP-1 (69),

all of which are known to be crucially involved in cellular activation and inflammation (70). Further, the increase in the levels of ROS can also cause oxidative modifications of lipids and proteins, thereby altering their functions. For example, the loss of superoxide dismutase (SOD; an important anti-oxidant defense enzyme) activity by oxidative modification has been shown in asthmatic airway epithelial cells (71), and has been evaluated as a surrogate marker of oxidant stress (57). Further, our recent study has shown that an important non-receptor tyrosine phosphatase, SHP-2, is a target of oxidation via an AhR- and ROS-dependent mechanism, leading to reduced activity of its phosphatase activity, and hence upregulating cellular activation in mast cells (49). Similarly, several studies have demonstrated that many key enzymes involved in cellular metabolism, including glycolysis, are targets of tyrosine nitration or S-nitrosylation (72), thereby reducing the activity or function of the parent proteins.

Moreover, increased oxidative stress response may lead to enhanced peroxidation of polyunsaturated fatty acids (PUFAs) and their metabolites. More than 20 lipoperoxidation end-products have been identified (73), including malondialdehyde (MDA), 4-hydroxyalkenals and isoprostanes (74). Importantly, MDA and 4-HNE are known to react with a variety of proteins, lipids and nucleic acids, and they are thought to have pro-inflammatory effect and contribute to the pathogenesis of human chronic diseases (75-77). An additional consequence of upregulated oxidative stress response involves the generation and liberation of lipid mediators, including prostaglandins, leukotrienes and lipoxins, in a tightly regulated, coordinated, cell- and context-specific manner (78). Lipid mediators are known to be important as initiators of pro- and anti-inflammatory responses (79,80). Recently, we found that AhR ligands could significantly up-regulate LTC_4 and LTB_4 production in mast cells, and that AhR signaling-induced ROS and AhR ligand's enhancing effect could be inhibited, at least in part, by inhibitors for 5-lipoxygenase (5-LO) and COX1, but not COX2, suggesting the possible functional role of eicosanoid metabolites in amplifying the effects of AhR ligands, for example, PAHs.

Conclusions and future challenges

Current epidemiological evidence from the study of various populations supports a link between air pollution and the development and exacerbation of asthma and allergic diseases. Also, recent advancement in the knowledge

about the effect of PM exposure clearly indicates its diverse impact on many different cell types at different levels of immune regulation. One intriguing and plausible hypothesis is that the expression and the increased prevalence of allergic diseases could be attributable directly to those environmental pollutants through their ability to increase the oxidant burden and inflammatory response or their immune "adjuvant" activities, and together contribute to the pathogenesis of asthma and allergic diseases. Thus, how tissue resident cells and immune regulatory cell types maintain the homeodynamic redox balance in response to environmental insult may represent an important mechanism in the genesis of, and/or the progression in, asthma and allergic diseases. Based on accumulated knowledge, *Figure 1* illustrates, schematically, a likely sequential event initiated following the exposure to environmental pollutants, leading to the expression of asthma.

While current assessment of air pollution's effect has been, understandably, limited to the evaluation of individual component, the highly dynamic "multi-pollutants effects" (81,82), which are expected to have additive or synergistic effects, will need to be examined in order to fully appreciate the extent to which the air pollution exerts its impact. Future effort will also be needed to use cutting-edge technology and analytical tools to dissect the complexity of air pollution and its interaction with other environmental risk factors, such as life style and eating habits, integrating the concept of "exposome" as originally proposed by Wild (83) to encompass life-course environmental exposures (including lifestyle factors) for better and more complete environmental exposure data for etiologic research (84). Mechanistically, additional studies will be needed to define the mechanisms by which air pollution (particularly PMs)-mediated oxidative stress impacts the expression of allergic diseases. Future efforts will also require "-omics" approaches, including metabolomics, to identify the disease relevant protein and lipid adducts and/or metabolites, as well as their functional consequences. Also, it has long been appreciated that PMs may trigger the alterations in autonomic nervous system leading to aberrant pulmonary function. Therefore, future effort in elucidating the responsible particle constituents and their underlying mechanisms will be needed.

The issue of genetic susceptibility to air pollution specifically and to the environment as a whole is another major challenge facing this field of investigation. So far, there has been no "genetic variant(s)" that can unequivocally define asthma and allergic diseases, and there have been no
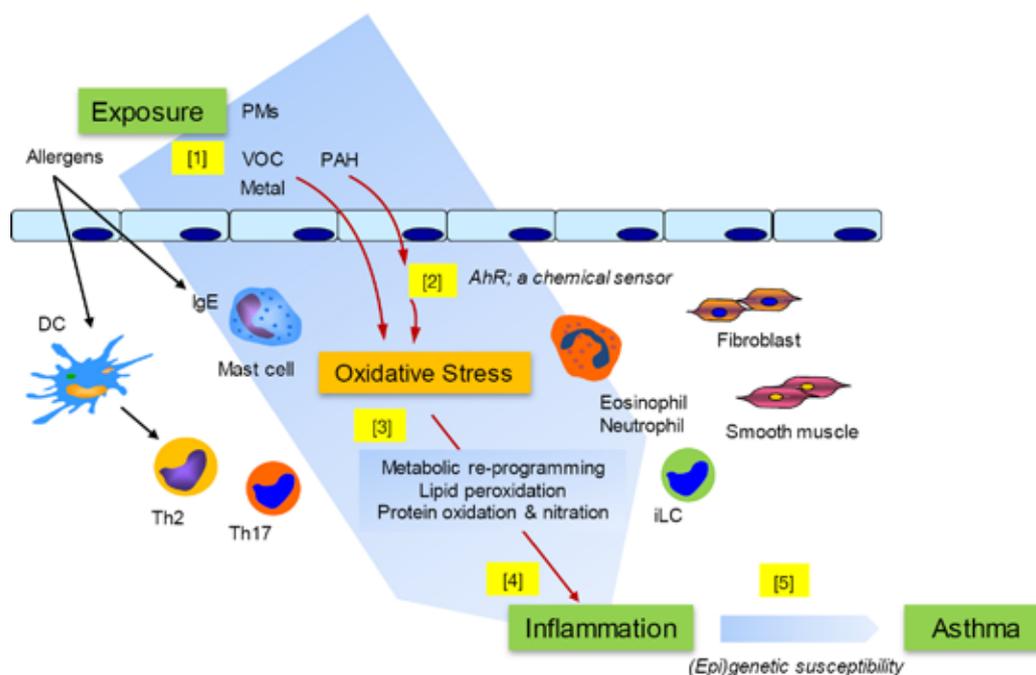
Figure 1 A mechanistic model of the exposure-disease relationship illustrating a sequential event originating from the exposure to the development of asthma. This model encompasses the major events known to be involved in asthma, including activation of pulmonary resident cells, including epithelium, fibroblasts and airway smooth muscle cells, as well as of those immune regulatory cell types, including dendritic cells (DCs), Th2, Th17, mast cells, granulocytes (eosinophils and neutrophils) and innate-type lymphoid cells (iLCs). They all could potentially be the target cell types and, collectively, contribute to the generation of oxidative stress and inflammatory responses. In this model, [1] the impact of air pollutants, including VOCs, metals and PAHs, either working alone or serving as immune adjuvant in conjunction with relevant allergens, is expected to be variable with pollutant-specific responses, but it appears to invariably converge on the induction of oxidative stress response either through their intrinsic oxidant-generating capacity, their ability to inhibit anti-oxidant activity or the combination thereof. [2] In this regard, the aryl-hydrocarbon receptor (AhR)-ligand (primarily PAHs) axis may provide a receptor-mediated mechanistic link between the exposure and the redox imbalance, as a part of a series of detoxification or bio-transformation of air pollutants, such as PAHs. [3] As the consequence of elevated ROS and RNS, concomitant with ER stress and mitochondrial dysfunction, changes in signaling events and a shift in cellular metabolism occur, resulting in the generation of metabolites, for example eicosanoids, and lipid peroxidation as well as modification of proteins by oxidation and/or nitration, some of which are able to induce, or amplify the existing, inflammatory response, either alone or play a role in conditioning the cells to become more sensitive to subsequent stimulation by pollutants and/or allergens. But, [4] any disturbance in the redox balance and/or homeodynamic state will result in the generation of pro-inflammatory mediators, including those involved in oxidative, metabolic and inflammatory responses, generated in each step of this sequential process, and [5] collectively, contribute to the development and exacerbation of the disease in genetically susceptible individuals and/or those with epigenetic modifications as the result of exposure to air pollutants.

genetic markers that are generally applicable in different ethnic backgrounds (85). Genetic heterogeneity and ethnical difference may all contribute to the failure to discover the bona fide "asthma gene(s)". More importantly, the issue of gene-environment interaction has not been properly addressed and has only been included in a relatively few genetic studies. Therefore, a critical challenge in the future is to investigate whether and how variations in the human

genome (polymorphisms) are able to modify the effect of exposures to environmental pollutants and chemicals or vice versa. It has been noted that the endogenous activity of the antioxidant mechanisms varies greatly between individuals, and that the ability to manage oxidative stress is altered in the lungs of asthmatics (86), leading to excessive ROS and its subsequent effects. In the future, the pursuit of this important area of research would ideally incorporate

the environmental-wide association studies (EWAS, or exposome-wide association studies), which has been fruitful in different disease context (87,88). It is relevant to note that in evaluating the features of the susceptible population to PM-related health effects, Sacks *et al.* (89) recently reported a group of characteristics, including life stage (i.e., children and older adults), preexisting cardiovascular or respiratory diseases, genetic polymorphisms and low-socioeconomic status, contributing to the increased risk of PM-related health effects.

In parallel, additional analyses utilizing powerful tools will be required for discovering and developing the next-generation of biomarkers to monitor the response to exposure, to capture the earlier pathologic events of inflammation and oxidative response as the consequence of exposure to environmental pollutants and chemicals, and to predict future clinically relevant events. For example, the fraction of exhaled NO (FeNO) (90,91), which has been used to distinguish asthma from non-asthma; but, it is a relatively non-specific marker of response and has, thus far, no value in defining a more specific disease phenotype and its severity. Also, the generation of lipid peroxidation products, including isoprostanoids and 4-HNE, as the result of the oxidative stress response has been evaluated (92,93). However, a major limitation with most of these parameters is their poor specificity and reproducibility when evaluating the disease progression and severity.

Recently, the developmental origin of human diseases has been an active field of investigation, wherein emerging evidence has suggested that gene-environmental interactions during pregnancy enhance the disease susceptibility through, in part, epigenetic mechanisms. This has been best studied in the context of cardiovascular and metabolic diseases, and the epidemic rise in chronic diseases, including allergic diseases, also highlights the likelihood of their maternal origin. These collective efforts will be critical in identifying important environmental risks, the time window of exposure and in defining the molecular mechanisms underlying the exposure-disease relationship. This should lead to better understanding of the role of environmental risk factors, including air pollution, in asthma and allergic diseases and ultimately to the development of better prevention strategies. Further, the outcomes of this collective effort are expected to provide crucial information for developing evidence-based interventional approaches and regulatory guidelines. Importantly, the success of this type of integrated approaches will help in improving public health awareness and ultimately the health of the general population, with a concomitant significant reduction in economic burden.

Footnote: *Initial assessment of the asthma burden in 2012 by Dr. Li-Kuan Chen, National Health Research Institutes, as a part of the "Consortium for Taiwan Asthma Study (CTAS)".

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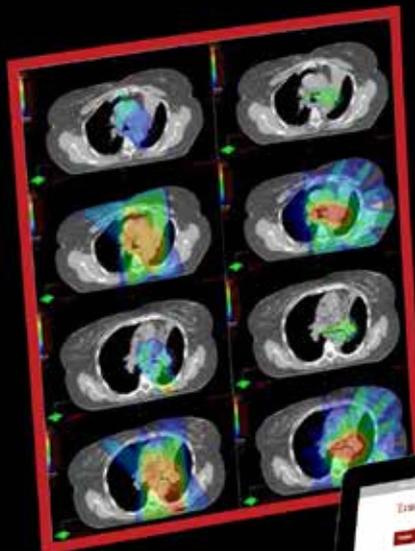
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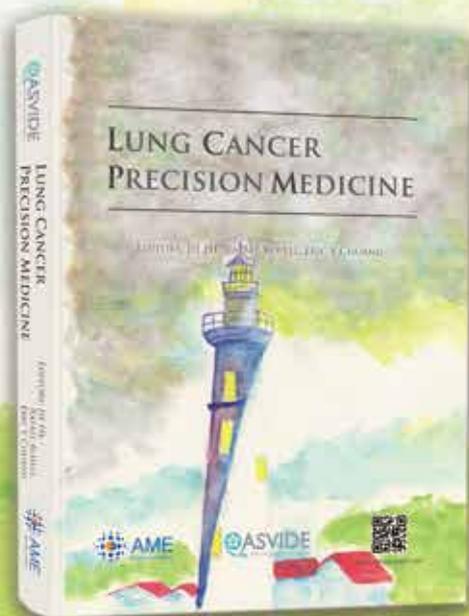
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