Editors: Dirk Van Raemdonck
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LUNG TRANSPLANTATION
IN THE THIRD MILLENNIUM

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LUNG TRANSPLANTATION IN THE THIRD MILLENNIUM (FIRST EDITION)

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Executive Typesetting Editor

We are pleased to announce that the "AME Research Time Medical Book Series" launched by AME Publishing Company has been published as scheduled.

Finishing my medical degree after 4 years and 3 months of study, I decided to leave the path toward becoming a doctor only after 3 months of training. After that, I had been muddling through days and nights until I started engaging in medical academic publishing. Even 10 years after graduation, I had not totally lost the affection for being a doctor. Occasionally, that subconscious feeling would inadvertently arise from the bottom of my heart.

In April 2011, Mr. Tiantian Li, the founder of DXY.cn, and I had a business trip to Philadelphia, where we visited the Mütter Museum. As part of The College of Physicians of Philadelphia, the museum was founded in 1858 and has now become an exhibition hall of various diseases, injuries, deformities, as well as ancient medical instruments and the development of biology. It displays more than 20,000 pieces of items including pictures of wounded bodies at sites of battle, remains of conjoined twins, skeletons of dwarfs, and colons with pathological changes. They even exhibited several exclusive collections such as a soap-like female body and the skull of a two-headed child. This museum is widely known as "BIRTHPLACE OF AMERICAN MEDICINE". Entering an auditorium, we were told by the guide that the inauguration ceremony of the Perelman School of Medicine at the University of Pennsylvania would take place there every year. I asked Mr. Li, "If it was at this auditorium that you had the inauguration ceremony, would you give up being a doctor?" "No," he answered.

In May 2013, we attended a meeting of the British Medical Journal (BMJ) and afterwards a gala dinner was held to present awards to a number of outstanding medical teams. The event was hosted annually by the Editor-in-Chief of the BMJ and a famous BBC host. Surprisingly, during the award presentation, the speeches made by the BMJ never mentioned any high impact papers the teams had published in any prestigious journals over the past years. Instead, they laid emphasis on the contributions they had made on improving medical services in certain fields, alleviating the suffering of patients, and reducing the medical expenses.

Many friends of mine wondered what AME means.

AME is an acronym of "Academic Made Easy, Excellent and Enthusiastic". On September 3, 2014, I posted three pictures to social media feeds and asked my friends to select their favourite version of the AME promotional leaflet. Unexpectedly, we obtained a perfect translation of "AME" from Dr. Yaxing Shen, Department of Thoracic Surgery, Zhongshan Hospital, Shanghai, who wrote: enjoy a grander sight by devoting to academia (in Chinese, it was adapted from the verse of a famous Chinese poem).

AME is a young company with a pure dream. While having a clear focus on research, we have been adhering to the core value of "Patients come first". On April 24, 2014, we developed a public account on WeChat (a popular Chinese social media social media platform) and named it "Research Time". With a passion for clinical work, scientific research and the stories of science, "Research Time" disseminates cutting-edge breakthroughs in scientific research, provides moment-to-moment coverage of academic activities and shares little-known behind-the-scene stories. With global vision, together we keep abreast of the advances in clinical research; together we meet and join our hands at the Research Time. We are committed to continue developing the AME platform to aid in the continual forward development and dissemination of medical science.

It is said that how one tastes wine indicates one's personality. We would say how one reads gives a better insight to it. The "AME Research Time Medical Books Series" brings together clinical work, scientific research and humanism. Like making a fine dinner, we hope to cook the most delicate cuisine with all the great tastes and aromas that everyone will enjoy.

Stephen Wang
Founder & CEO,
AME Publishing Company

After many attempts since the late '60s, lung transplantation has become a successful therapeutic option for a selected group of patients with end-stage respiratory failure since the early '80s. The evolution in the surgical technique, pre- and postoperative management with better immunosuppression, and appropriate donor selection further contributed during the following decades to improve the numbers and the results. Transplantation is a science where the future becomes past very quickly. From an era when the surgeon was forced to cover all the aspects of the transplant process, we have now reached a point where the pre-transplant candidate and the post-transplant recipient is followed by a multidisciplinary team, the key to long-term success.

In this text book, the editors have tried to cover all the aspects of lung transplantation, from the selection and management of donors to patient selection, intraoperative management and postoperative course, including potential complications.

We have also tried to open a window on the future, although future is already here; in fact the techniques of lung preservation, assessment, and reconditioning with machine perfusion are now becoming routine practice in many transplant centers, leaving hope for further development towards immunomodulation and regeneration of organs outside the body.

The authors involved in each chapter are experts in their fields. They have performed a wonderful job fulfilling their task and giving the readers a comprehensive overview on lung transplantation nowadays and a good inspiration for further studies.

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History of lung transplantation

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Abstract: Lung transplantation nowadays is a well-accepted and routine treatment for well selected patients with terminal respiratory disease. However, it took several decades of experimental studies and clinical attempts to reach this success. In this paper, we describe the early experimental activity from the mid-forties until the early sixties. The first clinical attempt in humans was reported by Hardy and Webb in 1963 followed by others with short survival only except for one case by Derom *et al.* who lived for 10 months. Long-term successes were not reported until after the discovery of cyclosporine as a new immunosuppressive agent. Successful heart-lung transplantation (HLTx) for pulmonary vascular disease was performed by the Stanford group starting in 1981 while the Toronto group described good outcome after single-lung transplantation (SLTx) for pulmonary fibrosis in 1983 and after double-lung transplantation for emphysema in 1986. Further evolution in surgical techniques and in transplant type for the various forms of end-stage lung diseases are reviewed. The evolution in lung transplantation still continues nowadays with the use of pulmonary allografts coming from living-related donors, from donors after circulatory death, or after prior assessment and reconditioning during ex vivo lung perfusion (EVLP) in an attempt to overcome the critical shortage of suitable organs. Early outcome has significantly improved over the last three decades. Better treatment and prevention of chronic lung allograft dysfunction will hopefully result in further improvement of long-term survival after lung transplantation.

Keywords: Lung transplantation; history; single-lung; double-lung; heart-lung

Lung transplantation nowadays is considered a viable therapeutic option for well selected patients with advanced respiratory disease. However, it took several decades of experimental studies and clinical attempts to reach this success. In fact, although the first human lung transplantation was performed more or less at the same time as other solid organs, with the exception of kidneys, its development to a routine procedure took longer than for other transplant types. The reasons for this delay were related to technical problems mainly involving healing of the bronchial anastomosis, to the high immunogenicity of the lung with need for immunosuppression, and the substantial risk of pulmonary infection.

Early experimental activity

The earliest attempt to transplant the heart and both lungs was performed by Alexis Carrel (*Figure 1A*) at the beginning of the 20th century; however, this experimental procedure involved only heterotopic transplantation of the heart–lung block into the neck of a recipient cat (1). In that model lung edema occurred with distention of the right side of the heart.

In the mid-forties the Russian surgeon Vladimir P.

Figure 1 Pioneers paving the way to human heart & lung transplantation. (A) Alexis Carrel at the time of his Nobel Prize presentation, 1912 (picture courtesy of the Nobel Foundation.); (B) Vladimir Demikhov (picture available at http://bored-bored.com/top-10-mad-scientists-in-history, accessed August 8, 2017); (C) Vittorio Staudacher; (picture courtesy by Dr. Luigi Santambrogio, Milan, Italy); (D) Henri Metras (picture available at http://patrimoinemedical.univmed.fr/rues/rues_metras.htm, accessed August 8, 2017).

Demikhov (*Figure 1B*) transplanted right lower lobes and the heart-lung block in dogs (2,3). At that time cardiopulmonary bypass was not yet available; Demikhov developed a technique to maintain blood supply to the brain continuously, with the exception of 2–3 minutes at one critical stage. The first animal recipient survived only for two hours without its organs; only after 1949 a more prolonged survival was obtained (3). Interestingly, the heart-lung block was kept viable during transfer from the donor to the recipient animal by closed—circuit circulation with the heart beating and blood pumped from the left ventricle into the aorta and coronary vessels and into the right atrium, the right ventricle and pulmonary circulation; by means of this ex situ circuit oxygenated blood was returned into the left atrium and adequate supply to the coronary circulation could be maintained. This preparation was the basis of means of transporting and temporary preserving the heart and lungs after harvesting during the first clinical attempts of heart–lung transplantation (4). The vascular and tracheal anastomoses were performed either by continuous suturing or by "quick connects" over prosthetic tubes. The caval veins were sutured separately; during inferior caval vein anastomosis the blood supply to the inferior part of the animal body was interrupted for 15–20 minutes. There were 67 attempts, but only 6 dogs survived for more than 48 hours and only 2 for more than 4 days. The dogs that recovered after surgery showed a slow respiratory rate and fast cardiac rate; some of them did remarkably well, being able to walk, drink and eat.

At that time other authors performed experimental heart-lung transplantation (HLTx). Marcus and coworkers developed a technique to transplant the heart and both lungs into the abdomen, giving the recipient two sets of heart and lungs; the purpose of their experiment was to determine if the heterologous block could be used as an extracorporeal pump during intracardiac operations or to temporary decrease the work load to the native heart (5,6). In 1950, Staudacher in Milan, Italy (*Figure 1C*) attempted canine lung transplantation using the right lower lobe and comparing for the first time autografts and allografts (7). Matejicek in 1956 reported a study on transplantation of the heart and the right upper lobe of the lung within the chest without mentioning any result (8).

Henri Metras in Marseille, France (*Figure 1D*) was the first to report in 1950 successful left lung transplantation in dogs (9). Interestingly, he was also the first to preserve and reimplant the bronchial arteries (to the subclavian artery) to immediately restore bronchial vascularization; he also described the use of a cuff of left atrium around the pulmonary veins to reduce the risk of venous thrombosis. In the early fifties, Juvenelle (10) and Neptune (11) performed lung autotransplantation in dogs to study the effects of denervation on lung physiology. In particular, Juvenelle in 1950 performed a pneumonectomy and reimplanted that lung by individual anastomosis of the bronchus and pulmonary artery and veins (10). Postoperative studies demonstrated vascular perfusion of the lung on the 18th day by angiocardiography and good lung function. Neptune performed 25 left lung allotransplants with no long-term survivors. Howard and Webb demonstrated nerve
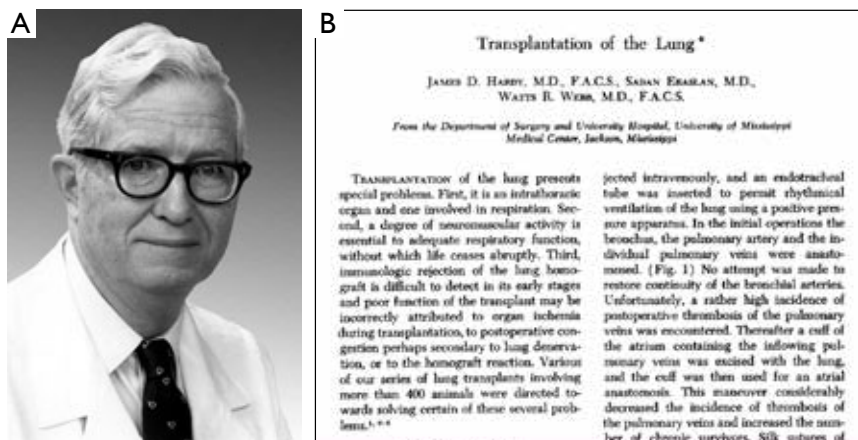
Figure 2 The first human lung transplantation was performed on June 11, 1963 at the University Hospital, Jackson, Mississippi by James D Hardy and his team. The donor died of massive heart attack resulting in heart failure and shock and was therefore the first non-heart-beating donor (NHBD), now named donor after circulatory death (DCD). A left SLTx was successfully performed. The recipient, the 58-year-old John Russell with an obstructive tumor in the left main bronchus died 18 days later from renal failure. (A) James D. Hardy (picture available at https://www.umc.edu/som/Departments%20and%20Offices/SOM%20Departments/Surgery/Residents/About-Us/James%20Hardy/James-Hardy.html, accessed August 8, 2017); (B) Original paper as published in *Ann Surg* presented at the Annual Meeting of the American Surgical Association, Hot Springs, Virgina, April 1–3, 1964 (18).

regeneration microscopically (12,13). In 1954 Hardin and Kittle reported their results with left lung allografts in dogs using the same atrial cuff technique as previously reported by Metras (14). This is the first report on living-related lung transplantation since in three transplants, donors were litter-mates. Survival was between 1 and 12 days, but it was 30 days in the living-related transplants. Pneumonia was the most frequent cause of death. In two cases they performed contralateral pneumonectomy immediately after the transplant; the animals survived 6 and 9 days proving the effective function of the pulmonary allograft.

In 1961 Blumenstock and Kahan demonstrated that methotrexate was helpful to increase allograft survival in dogs (15).

Early clinical experience

After 7 years of experimental research on hundreds of animals, Hardy and Webb at the University of Mississippi, Jackson obtained permission to perform lung transplantation in a human recipient with the following characteristics: the patient should have a potentially fatal disease; there should be a reasonable chance that he would benefit from the transplant; the removal of the patient's own lung should not result in the sacrifice of a significant amount of his own functioning lung parenchyma; the

transplant would have been performed on the left side, since it was judged to be technically easier (16).

On April 15, 1963, a 58-year old man with left lung bronchial carcinoma was admitted at the University of Mississippi Hospital. He showed dyspnea at rest from advanced emphysema; he had borderline renal failure due to chronic glomerulonephritis. He also had obstructive pneumonia due to neoplastic occlusion of the left main bronchus. The indication for pneumonectomy was based on the proximal location of cancer within the bronchus; the impossibility to perform curative left pneumonectomy was related to respiratory failure; the indication for left lung transplantation was consequent (17).

At approximately 7.30 pm on June 11, 1963, a patient entered the emergency room of the same hospital in shock due to massive myocardial infarction. All resuscitative efforts failed and the family allowed donation of the left lung for transplantation. This was the first non-heart beating donor used for lung transplantation. Left lung transplantation was performed successfully by James D. Hardy and his team (*Figure 2*). The new lung worked properly for the subsequent 18 days. At that time immunosuppression was based on the administration of azathioprine and prednisone, and it included mediastinal irradiation. The patient survived 18 days and died of renal failure and infection. At autopsy, the lung was well ventilated and there was no evidence
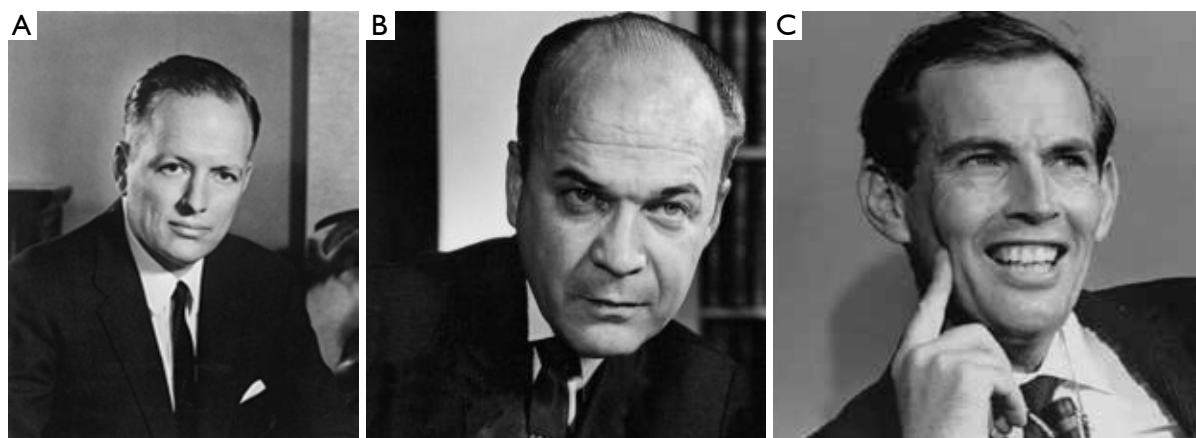
Figure 3 Three pioneers of human heart-lung transplantation. (A) Denton Cooley, Houston, Texas, 1968 (picture available at http://www. hopkinsmedicine.org/news/media/releases/pioneering_heart_surgeon_denton_a_cooley_dies_at_96, accessed August 8, 2017); (B) Walton Lillehei, University of Minnesota, Minneapolis, 1969 (picture available at http://www.thefamouspeople.com/profiles/clarence-walton-lillehei-5381.php, accessed August 8, 2017); (C) Christian Barnard, Grote Schuur Hospital, Cape Town, South Africa, 1971 (picture available at https://alchetron.com/Christiaan-Barnard-750845-W, accessed August 8, 2017).

of rejection; the vascular anastomoses were patent; the bronchus showed a small defect on the membranous side, but it was sealed by the inflammatory reaction of the surrounding tissues (18).

On August 31, 1968, Denton Cooley (*Figure 3A*) performed in Houston, Dallas the first HLTx in a 2-month old infant with a complete atrioventricular canal defect, pulmonary hypertension, and pneumonia (19). The child was reoperated for bleeding and died 14 hours after the transplant. The second HLTx was performed by Walton Lillehei (*Figure 3B*) in December 1969, in a 43-year-old patient with emphysema and pulmonary hypertension; he survived 8 days and died of pneumonia (20). The third HLTx was performed in July, 1971 in Cape Town, South Africa by Christian Barnard (*Figure 3C*) (21), who previously reported the first heart transplant (22). At that time airway anastomosis was performed at the level of the bronchi, in an attempt to preserve blood supply to the recipient carina and cough reflex. This latter patient died on the 23rd postoperative day with a bronchopleural fistula on the right side. Barnard performed a pneumonectomy on this side, but fatal septicemia eventually occurred.

Between 1963 and 1978 approximately 38 lung, lobe or HLTx had been attempted, with no long-term success (23). Only one patient was discharged from the hospital in 1968 (23), and only nine lived more than two weeks (24). The vast majority died in the third postoperative week from disruption of the bronchial anastomosis. For this reason

interest in lung transplantation decreased for most of the 1970s. The only medium-term survival was in a patient transplanted by F. Derom in Ghent, Belgium (*Figure 4A*) on November 14, 1968 (25). The recipient was a 23-year-old man with silicosis (*Figure 4B*). Left lung transplantation was performed (*Figure 5*). He died of bronchopneumonia in the transplanted lung after 10 months; at histology there were no signs of acute rejection, but some lesions were compatible with chronic rejection. Since the right lung was completely fibrotic and shrunken, we can suppose that the patient had lived at least the last months on the grafted lung.

In 1978 the group in Toronto led with Joel Cooper performed right lung transplantation in a young man suffering of respiratory burns due to a house fire requiring mechanical lung ventilation (27). At that time membrane oxygenators were available at that center (28) and the surgeons believed that this support could improve the chances of success. That patient, notwithstanding maximum ventilatory assistance, had a pCO_2 of 130 mmHg and required the use of the membrane oxygenator before, during and for four days after the procedure. Interestingly, the transplanted lung was pretreated with cytotoxic drugs. Within two weeks after the transplant the ventilatory system could be discontinued and the patient become ambulatory. The patient died in the third postoperative week of disruption of the bronchial anastomosis.

At that time it became clear that the major determinant for the future success of lung transplantation was related to
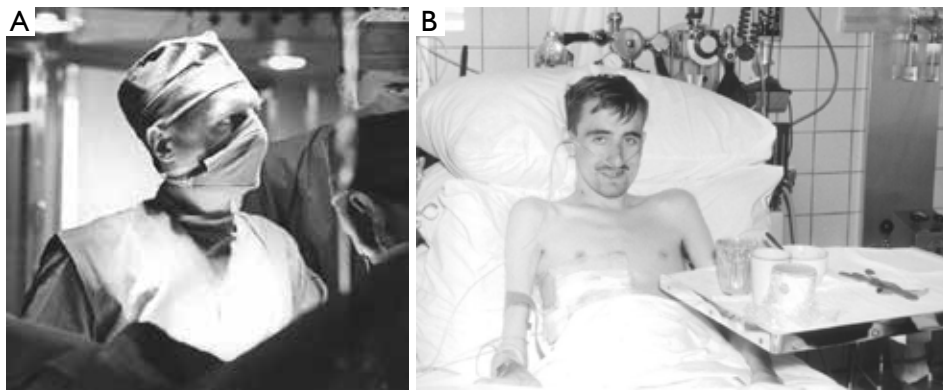
Figure 4 Lung transplantation: first mid term survivor in Belgium. (A) Prof. em Dr. Fritz Derom who performed a single-lung transplantation at the University Hospital in Ghent, Belgium on 14th November 1968; (B) the patient Aloïs Vereecken became the first mid-term (10 months) survivor at the time (25). Pictures available at http://www.ugentmemorie.be/gebeurtenissen/1968-wereldprimeur-longtransplantatie, accessed August 8, 2017.

Figure 5 A right single-lung transplantation performed by Prof. em Dr. Fritz Derom at the University Hospital in Ghent, Belgium on 14th November 1968 in patient Aloïs Vereecken who suffered from end-stage lung fibrosis (silicosis resulting from professional sand-blasting) (26); (permission for reprint given by Fritz Derom at age of 90 years).
Available online: http://www.asvide.com/articles/1908

the establishment of an adequate bronchial arterial supply to the transplanted bronchus and the prevention of bronchial necrosis and disruption.

Back to the lab

The group in Toronto decided not to embark on further lung transplantation until they had gained better understanding of the cause of bronchial disruption and the means to prevent it. This is the reason why most of

the animal laboratory research at that time has been done at their laboratories. Three factors were considered to be potentially related to bronchial dehiscence: rejection, ischemia (the lung is the only organ without systemic arterial vascularization routinely reimplanted at the time of transplantation), and immunosuppressive drugs (prednisone and azathioprine at that time).

The first series of animal experiments was dedicated to evaluate the potential role of rejection. These were based on autotransplantation of the lung in dogs. With this model factors associated with rejection were obviously eliminated. Half of the animals did not receive any immunosuppression while the other half received the same immunosuppressive regimen as if allotransplantation would be performed (29). Animals receiving no immunosuppression showed good primary healing of the anastomosis (only a degree of distal narrowing) while those receiving prednisone and azathioprine had the same incidence of bronchial complications as it had been observed in human transplants. Furthermore, in this group of animals, the breaking strength of the anastomosis was markedly reduced. At that time cyclosporine become available and the same experiment was repeated administering this drug instead of the association of prednisone and azathioprine (30). With cyclosporine, wound healing and breaking strength were not impaired. In subsequent studies prednisone and azathioprine were evaluated separately and the former was clearly responsible for the inhibition of bronchial healing (31).

Bronchial narrowing distal to the suture line was believed to be related to ischemia. To address this problem, the authors

Figure 6 The first long-term success after human heart-lung transplantation was performed by Bruce Reitz and colleagues at Stanford University, Palo Alto, California, on March 9, 1981 (34). (A) Bruce Reitz; (B) picture taking in the operating room during transplantation. (pictures Stanford School of Medicine available at https://twitter.com/StanfordMed/status/707709259778269184, accessed August 8, 2017).

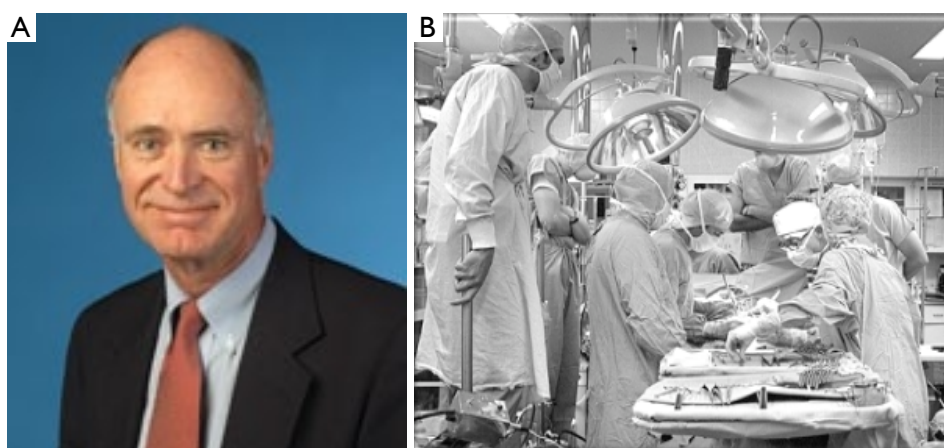used the greater omentum. This organ was known for its ability to bring rapidly new blood supply when transposed into a potentially ischemic area. A new set of experiments were performed bringing the omentum into the chest and wrapping it around the bronchial anastomosis (32,33). The animals were autopsied at various intervals after transplantation. In all the animals collateral circulation between the omentum and the airway at the level of the anastomotic site was observed. Normally, it takes between 2 and 3 weeks to restore a sufficient bronchial circulation by means of vascular growth.

Thus, changing the immunosuppressive regimen with the introduction of cyclosporine and protecting the bronchial anastomosis with a wrap of omentum, would certainly have contributed to prevent the dreadful complications that affected all the transplants previously performed.

The new era

A review of the literature showed that most of the previous clinical attempts were performed under relatively adverse circumstances: the patients were often debilitated, infected, with multisystem problems, ventilated and even on ECMO. It was believed that results could be improved by careful patient selection; those with limited life expectancy were certainly suitable candidates, but they should not yet have become hospital-dependent or ventilator-dependent.

On March 9, 1981 Bruce Reitz and his colleagues at Stanford University performed the fourth HLTx (*Figure 6*). This patient was the first long-term survivor, thanks to the improved surgical technique and the use of

cyclosporine. The patient was a 45-year old woman with primary pulmonary hypertension (PPH). She suffered two acute rejection episodes successfully reversed, with a good lung function preserved for a long time (35). Two more patients underwent the same operation during the following 4 months (34).

The selection of candidates for single-lung transplantation (SLTx) was much more difficult. The group in Toronto believed (*Figure* 7) that idiopathic pulmonary fibrosis (IPF) was the ideal disease; these patients generally do not have pulmonary sepsis; furthermore, the reduced compliance and the increased vascular resistance of the native, fibrotic lung would help ensure that ventilation and blood flow (perfusion) will be diverted to the transplanted lung after SLTx.

In 1983, a 58-year-old patient with IPF with the above mentioned characteristics inquired for the possibility of a transplant in Toronto. He was on oxygen 24 hours a day with a limited life expectancy. He underwent right lung transplantation on November 7, 1983; the omentum was transposed into the chest and wrapped around the bronchial anastomosis and tacked to itself. At that time the vascular anastomoses were performed before suturing the bronchus, in order to reduce the ischemic time. He was discharged in 6 weeks and returned to work in 3 months. He died more than 7 years later of renal failure (36). The group at Freeman Hospital in Newcastle-upon-Tyne, U.K. followed the path shown by the Toronto group and reported similar good outcome in patients with pulmonary fibrosis (37).

At that time there were two other axioms that were
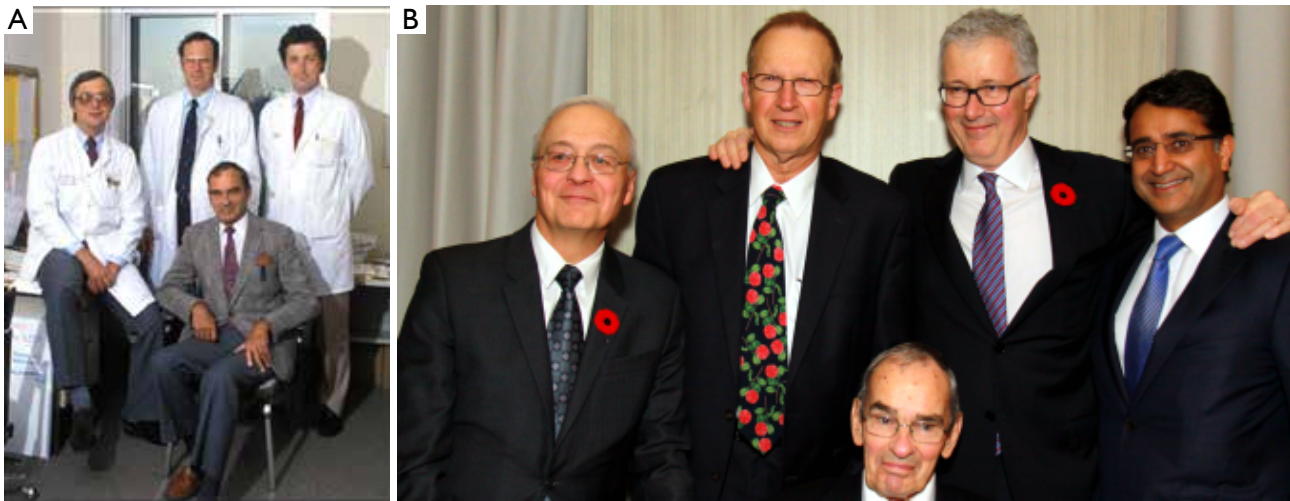
Figure 7 The first long-term success after isolated lung transplantation in patient Tom Hall was performed by the Toronto Lung Transplant Group, Toronto, Ontario, Canada on November 7, 1983 (36). (A) Picture taken in 1987. Surgeons depicted: standing (from left to right): Thomas R. Todd, Joel D. Cooper, G. Alexander Patterson; sitting: F. Griffith Pearson; (B) The Toronto Lung Transplant Group at the 30th anniversary celebration of the first long-term success after single-lung transplantation. The group is now headed by Shaf Keshavjee (same surgeons as depicted in A with Shaf Keshavjee standing fare right (pictures available at http://www.imsmagazine.com/pioneering-lung-transplantation-in-toronto/, accessed August 8, 2017).

subsequently unhitched: (I) SLTx in patients with chronic obstructive pulmonary disease (COPD) was considered not feasible due to concern related to the potential overinflation of the contralateral lung with displacement of the mediastinum toward the transplanted lung; (II) patients with pulmonary sepsis required HLTx, since isolated transplantation of both lungs without the heart was not yet technically feasible.

Cooper and his colleagues believed that most of the patients with septic lung disease have adequate or recoverable right heart function and, at least theoretically, they do not require replacement of the heart. This was confirmed by the pathological reports on the heart removed at that center during HLTx, supporting the idea that the morphology and the function of the heart was preserved. Inclusion of the heart as part of the transplantation procedure was mandated primarily for technical reasons and not because of physiologic necessity. Further confirmation of this feeling came by the "domino" procedure subsequently performed at many centers: the native heart removed from the lung recipient at the time of HLTx was then allotransplanted in another patient with heart failure with excellent postoperative function (38,39). However, at that time HLTx was considered the procedure of choice also to preserve airway vascularization after tracheal anastomosis

as small branches of the coronary circulation through the pericardium supply the subcarinal bronchial vessels and maintain tracheal circulation.

Further experiments were conducted to evaluate the possibility to perform isolated bilateral lung transplantation; simultaneous bilateral lung transplantation was proposed by Grosjean and coauthors (40,41). En bloc double-lung transplantation (DLTx) was initially proposed by Vanderhoeft and coworkers in 1972 (42). The experimental activity was initially performed in dogs; however, these animals are unable to breathe after bilateral pulmonary denervation; thus, primate models were required. Feasibility of simultaneous double-lung transplantation was demonstrated in primates in the laboratory at the University of Toronto (43), and it was subsequently performed, on November 26, 1986, in a 42-year old patient with end-stage emphysema (44). The procedure was conducted through median sternotomy, under cardioplegic arrest and full cardiopulmonary bypass, with a tracheal anastomosis wrapped with the transposed omentum, an anastomosis between the donor atrial cuff and the back of the recipients' left atrium, and an anastomosis between the main pulmonary artery of the donor and the recipient. It was a technically demanding procedure. Furthermore, further experience with this procedure demonstrated a significant

incidence of airway complications at the level of the tracheal anastomosis (45). For this reason, during the following years, the procedure was progressively simplified. To avoid problems with tracheal anastomosis, research on bronchial artery revascularization was conducted (46) and several attempts were clinically performed in Copenhagen (47), Bordeaux (48), and later in Cleveland (49), but the technique never gained wide acceptance worldwide. Michel Noirclerc in Marseille (50) proposed to move the tracheal anastomosis to the level of the main bronchi. To avoid the need for CPB, reduce surgical trauma, and improve results, the Group in St Louis, with Joel Cooper, Alec Patterson and Michael Pasque performed double-lung transplantation as two separate sequential single-lung transplant (51,52). They used the old-fashioned clamshell incision (bilateral thoracosternotomy) to enter both pleural cavities simultaneously, transplanting first on the side of the worst lung. Bisson & Bonette in Paris developed this bilateral procedure at the same time (53). The clamshell incision was progressively abandoned at most of the centers because of problems with wound healing, and substituted by two anterolateral thoracotomies, avoiding sternal transection (54,55).

At the same time when improved techniques for bilateral lung transplantation (BLTx) were developed, the indications for SLTx expanded. After the initial report from the group of Andreassian in France (56-58), SLTx has been successfully performed in patients with emphysema by other groups (59,60).

In the mid-1990s SLTx was used for a selected group of patients with PPH. This procedure was encouraged by previous reports from a similar patient population: patients with pulmonary hypertension undergoing thromboendarterectomy for chronic pulmonary embolism had favorable hemodynamic response despite compromised preoperative right ventricular function (61). Experimental studies supported this idea (62). The successful clinical experience of the group in St Louis confirmed the value of the procedure (63,64), although sequential BLTx was subsequently considered a better option compared to SLTx for patients with pulmonary vascular disease to avoid life-threatening complications resulting in early (reperfusion edema) and late (bronchiolitis obliterans) graft dysfunction and also compared to HLTx because of the critical shortage for donor hearts (65).

Intraoperative cardiopulmonary support

Cardiopulmonary support is often needed in lung transplantation, especially in patients with pulmonary hypertension or with disturbances in gas exchange upon clamping of the first native lung prior to extraction (severe hypoxia or inappropriate ventilation). Historically, conventional cardiopulmonary bypass (CPB) was used to circumvene these intra-operative problems and is still used by many lung transplant centers worldwide. Discussion on the need for routine use of CPB is still ongoing (66,67). More recently, a shift has been noticed towards the use of veno-arterial extracorporeal membrane oxygenation (V-A ECMO) to decompress the right ventricular preload and to improve gas exchange intraoperatively. The advantages of V-A ECMO over CPB are the reduced need for anticoagulation with less bleeding and a lower inflammatory response resulting in less postoperative complications. The Vienna group was the first to describe the use of V-A ECMO for intra-operative support (68). Many more groups worldwide have now switched from CPB to V-A ECMO with superior early outcomes reported (69-72).

The future is here

Many problems still need to be solved. During the last decade, the number of transplants around the world remained substantially stable after a previous exponential increase (73). This was mainly related to the limited availability of suitable organs for transplantation. For this reason extended-criteria donors not fulfilling the standard criteria are increasingly being used (74-76). However, there are other means to increase the total number of lung donors:

(I)   Living-related transplants;
(II) Lungs from donation after circulatory death donors (DCD's);
(III) Ex vivo evaluation and reconditioning of previously unsuitable donor lungs.

Living-related lung transplantation

Living-donor lobar lung transplantation (LDLLT) was successfully introduced by Vaughn Starnes and his colleagues at the University of Stanford in the late 1990 (77). The first procedure was performed transplanting a mother's right upper lobe to her 12-year old daughter born with bronchopulmonary dysplasia and the patient survived. The second patient was a 3-year old girl with Eisenmenger's syndrome. Starnes performed again a right single-lobe transplant using the middle lobe of his father and he also closed the ventricular septal defect.

Unfortunately this second patient died of primary graft dysfunction. After this unsuccessful experience, the group switched to bilateral LDLLT, harvesting the lower lobes from two healthy living donors (78,79). The small volume of the two transplanted lobes encouraged to perform this procedure only in children, at least at the beginning of the historical experience; it was applied almost exclusively to cystic fibrosis patients (80). However, it is now recognized that it can be applied also to patients with obstructive, restrictive, infectious and hypertensive lung disease, both in the pediatric and adult population given an acceptable size matching (81,82). Although LDLLT was initially proposed in the United States, the number of cases performed in that country progressively decreased due to the changes of organ sharing favoring an urgency benefit for allocation of cadaveric organs. For this reason, reports of LDLLT came almost exclusively from Japan, where the average waiting time for a cadaveric lung is more than 2 years (82). Other small series were published from Brazil (83) and China (84). Overall, the results of LDLLT are equal if not better than conventional cadaveric lung transplantation (85). Currently, the group at Kyoto University led by H. Date is continuing to pioneer this procedure with excellent results in a difficult group of patients.

Lungs from donation after circulatory death donors (DCD's)

The very first lung transplantation in humans by James Hardy in 1963 was performed with a pulmonary graft coming from a donor who died after myocardial infarction (17). The donor lung therefore was first subjected to a period of warm ischemia prior to topical cooling. After acceptance of the Harvard criteria on brain death (86), the practice of using organs from donors dying from cardiocirculatory arrest (DCD's), previously called non-heart-beating donors (NHBD's) (87), was largely abandoned. Following an international workshop organized on 30–31 March 1995 by Kootstra in Maastricht, the Netherlands, different categories of DCD's have been recognized based on the so called Maastricht classification (88).

The concept of lung transplantation from DCD's was reintroduced by Thomas M. Egan (Chapel Hill, NC, USA) in 1991 following a series of dog experiments (89). This concept was based on the fact that lung tissue may remain viable for a certain period after death as a result of the oxygen reserve present in the alveoli. His work has stimulated many research groups worldwide to explore the possibility of transplanting lungs from donors after circulatory arrest (90).

In 1995, Robert Love (Madison, WI) reported a successful first case report following transplantation of a single lung from a controlled DCD (91). In 2001, Stig Steen (Lund, Sweden) reported a remarkable case of successful SLTx from an uncontrolled DCD after pretransplant *ex vivo* perfusion for functional evaluation (92). Most reported series on DCD lung transplantation nowadays come from groups retrieving organs from donors with severe brain damage after withdrawal of life support (DCD Maastricht Category III). Short and long-term outcome in lung recipients from such donors have been reported to be equal to brain-dead donors in experienced centers (93). This was confirmed in a recent meta-analysis of reported observational cohort studies (94) and in a published collective series of 306 transplants submitted to the International Society for Heart and Lung Transplantation DCD Registry (95). The group headed by A. Varela in Madrid, Spain reported in 2004 two successful lung transplants from uncontrolled DCD's (Maastricht Categories I and II) after failed resuscitation in the donor (96). In subsequent papers, the incidence of primary graft dysfunction grade 3 was reported to be higher with lower short-term survival in these recipients. This underlines the need to evaluate pulmonary graft performance with the use of *ex vivo* lung perfusion (EVLP) prior to transplantation (97). The world's experience with uncontrolled donors so far remains anecdotal with successful case reports by individual centers only (98,99).

Ex vivo evaluation and reconditioning of previously unsuitable lungs

As previously mentioned, Stig Steen in Lund, Sweden was the first to report a successful case of SLTx from a DCD after *ex vivo* evaluation of the pulmonary graft (92). Steen introduced the concept of EVLP as a method, not only to evaluate more objectively lung performance prior to transplantation, but also as a possible technique to revitalize donor lungs of inferior quality outside the body previously regarded as unusable for transplantation (100). This new technique may open new perspectives in the field of lung transplantation in the future (101).

Early attempts to perfuse lungs were complicated by edema formation and deterioration of pulmonary function (102,103). A fundamental step towards success was the development of Steen Solution™, a buffered perfusate with high albumine concentration to create an ideal colloid

osmotic pressure of about 30 mmHg allowing physiologic perfusion pressures and flow to be maintained without the development of either tissue edema or dehydration (104). Clinical EVLP was pioneered by the groups in Lund and Toronto. A first prospective clinical case series (HELP trial) was published in a landmark publication by Cypel and Keshavjee from Toronto in 2011 showing non-inferiority for PGD and 30-day mortality in 20 EVLP lung transplant recipients compared to 116 contemporaneous standard lung transplants performed in their center (105). Since then, many other groups around the world have reported similar experience with transplantation of EVLP lungs initially rejected for transplantation with a utilization rate between 39–100% (101). EVLP nowadays is becoming a standard technique for better assessment of questionable lungs from extended-criteria donors and from DCD's with uncertainty about their post-transplant function (106). The outcome in EVLP lung recipients is now further investigated in prospective clinical trials (NOVEL trial, EXPAND trial). Anecdotal case reports were published on successful transplantation of previously unacceptable and injured lungs after drug treatment during EVLP (107-109). If longer perfusion times (>12–24 hours) would prove to be possible, repair of injured grafts by delivered therapies interacting via several mechanisms could be investigated. Several treatment pathways have been suggested including the administration of infused or inhaled drugs, gases, and stem cell therapy (110). Further research is needed in order to maximize the yield of donor lungs for transplantation. Hopefully one day we will be able to repair pulmonary allografts in a box to become "brand-new lungs" prior to implantation (111).

Different protocols and techniques for static EVLP have been described and several commercial EVLP devices have now become available for clinical use (101). In addition, when the final results of the Inspire trial prove to be favourable, dynamic normothermic EVLP with a portable device may become a new preservation method for standard donor lungs thereby reducing the cold ischemic period and safely prolonging the cross clamp time making the transplantation process a better planned procedure (112).

Conclusions

Lung transplantation has come a long way since the first human lung transplantation by J Hardy in 1963 and the first clinical successes in the early eighties. After more than fifty years of clinical experience, lung transplantation in many experienced centers across the world has now become a standard and routine procedure for patients with end-stage lung disease with no other treatment options available. Better early and late survival observed over the years is multifactorial and is expected to further improve in the third millennium. History will learn whether we will be able to overcome the persistent hurdles of donor organ shortage and chronic lung allograft dysfunction. Hope remains that recipient morbidity resulting from life-long immunosuppressive therapy one day will be overcome with immunomodulatory techniques resulting in better tolerance of the allograft.

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Footnote

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References

1. Carrel A. The surgery of blood vessels. Johns Hopkins Hosp Bull 1907;18:18-28.
2. Demikhov VP. Transplantation of the heart, lungs and other organs. Eksp Khir Anesteziol 1969;14:3-8.
3. Becerra E, Kaplan J, Cooper DKC. Experimental background and early clinical experience. In: The transplantation and replacement of thoracic organs. Ed Cooper DKC and Novitzky D. Dordrecht/Boston/London: Kluwer Academic Publishers, 1990;Cap 33:261-72.
4. Robicsek F, Pruitt JR, Sanger PW, et al. The maintenance of function of the donor heart in the extracorporeal stage and during transplantation. Ann Thorac Surg 1968;6:330-42.
5. Marcus E, Wong SN, Luisada AA. Homologous heart grafts: transplantation of the heart in dogs. Surg Forum 1951;2:212-7.
6. Marcus E, Wong SN, Luisada AA. Homologous heart grafts. I: Technique of interim parabiotic perfusion. II: Transplantation of the heart in dogs. AMA Arch Surg 1953;66:179-91.
7. Staudacher VE, Bellinazzo P, Pulin A. Primi rilievi su tentativi di reimpianti autoplasici e di trapianti omoplasici di lobi polmonari. Chirurgia 1950;5:223-8.
8. Matejicek E. Transplantation of organs. Transplant Bull

1956;3:167.

9. Metras H. Note preliminaire sur greffe totale du poumon chez le chien. Comp Renal Acad Sci 1950;231:1176-7.

10. Juvenelle AA, Citret C, Wiles CE, et al. Pneumonectomy with the reimplantation of the lung in the dog for physiologic study. J Thorac Surg 1951;21:111-5.

11. Neptune WB, Weller R, Baily CP. Experimental lung transplantation. J Thorac Surg 1953;26:275-89.

12. Howard HS, Webb WR. Respiratory paralysis following pulmonary denervation. Surg Forum 1957;8:466-9.

13. Webb WR, Howard HS. Cardiopulmonary transplantation. Surg Forum 1957;8:313-7.

14. Hardin CA, Kittle CF. Experience with transplantation of the lung. Science 1954;119:97-8.

15. Blumenstock DA, Kahan DR. Replantation and transplantation of the canine lung. J Surg Res 1961;1:40-7.

16. Hardy JD. Cap 45: Experimental background and early clinical experience. In: Cooper DK, Novitzky D. editors. The Transplantation and Replacement of Thoracic Organs. Dordrecht: Springer, 1990:357-61.

17. Hardy JD, Webb WR, Dalton ML Jr, et al. Lung homotransplantation in man. JAMA 1963;186:1065-74.

18. Hardy JD, Araslan S, Webb WR. Transplantation of the lung. Ann Surg 1964;160:440-8.

19. Cooley DA, Bloodwell RD, Hallman GL, et al. Organ transplantation for advanced cardiopulmonary disease. Ann Thorac Surg 1969;8:30-46.

20. Wildevuur CR, Benfield JR. A review of 23 human lung transplantations by 20 surgeons. Ann Thorac Surg 1970;9:489-515.

21. Losman JG, Campbell CD, Reploge RL, et al. Joint transplantation of the heart and the lungs. Past experience and present potentials. J Cardiovasc Surg (Torino) 1982;23:440-52.

22. Barnard CN, Cooper DKC. Clinical transplantation of the heart: a review of 13 years personal experience. J Roy Soc Med 1981;74:670-4.

23. Cooper JD. Lung transplantation. Ann Thorac Surg 1989;47:28-44.

24. Wildevuur CRH, Benfield JR. A review of 23 human lung transplantations by 20 surgeons. Ann Thorac Surg 1970;9:489-515.

25. Derom F, Barbier F, Ringoir S, et al. Ten month survival after lung transplantation in man. J Thorac Cardiovasc Surg 1971;61:835-46.

26. Venuta F, Van Raemdonck D. A right single-lung transplantation performed by Prof. em Dr. Fritz Derom at the University Hospital in Ghent, Belgium on 14th November 1968 in patient Aloïs Vereecken who suffered from end-stage lung fibrosis (silicosis resulting from professional sand-blasting). Asvide 2017;4:583. Available online: http://www.asvide.com/articles/1908

27. Nelems JM, Rebuck AS, Cooper JD, et al. Human lung transplantation. Chest 1980;78:569-73.

28. Nelems JM, Duffin J, Glynn F, et al. Extracorporeal membrane oxygenator support for human lung transplantation. J Thorac Cardiovasc Surg 1978;76:28-32.

29. Lima O, Cooper JD, Peters WJ, et al. Effects of methylprednisolone and azathioprine on bronchial healing following lung autotransplantation. J Thorac Cardiovasc Surg 1981;82:211-5.

30. Goldberg M, Lima O, Morgan E, et al. A comparison between cyclosporine A and methylprednisolone plus azathioprine on bronchial healing following canine lung allotransplantation. J Thorac Cardiovasc Surg 1983;85:821-6.

31. Saunders NR, Egan TM, Chamberlain D, et al. Cyclosporine and bronchial healing in canine lung transplantation. J Thorac Cardiovasc Surg 1984;88:993-9.

32. Dubois P, Choiniere L, Cooper JD. Bronchial omentopexy in canine lung allotransplantation. Ann Thorac Surg 1984;38:211-4.

33. Morgan E, Lima O, Goldberg M, et al. Successful revascularization of totally ischaemic bronchial autografts in dogs using omental pedicle flaps. J Thorac Cardiovasc Surg 1982;84:204-10.

34. Reitz BA, Wallwork J, Hunt SA, et al. Heart and lung transplantation: successful therapy for patients with pulmonary vascular disease. N Engl J Med 1982;306:557-64.

35. Reitz BA, Pennock JL, Shumway NE. Simplified operative method for heart and lung transplantation. J Surg Res 1981;31:1-5.

36. Toronto Lung Transplant Group: unilateral lung transplantation for pulmonary fibrosis. N Engl J Med 1986;314:1140-5.

37. McGregor CG, Dark JH, Hilton CJ, et al. Early results of single lung transplantation in patients with end-stage pulmonary fibrosis. J Thorac Cardiovasc Surg 1989;98:350-4.

38. Shudo Y, Ma M, Boyd JH, et al. Current status of domino heart transplantation. J Card Surg 2017;32:229-32.

39. Cooper JD. Dominoes – pragmatism or piracy? Transpl Int 1991;4:1-2.

40. Grosjean O, Noirclerc M, Duvinage JF, et al. Bilateral lung transplantation. Preliminary report. Acta Chir Belg

1972;Suppl 2:49-55.

41. Grosjean O, Noirclerc M, Mattei M, et al P. One-stage bilateral allogenic pulmonary transplantation. Ann Chir Thorac Cardiovasc 1973;12:105-8.

42. Vanderhoeft P, Dubois A, Lauvau N, et al. Block allotransplantation of both lungs with pulmonary artery trunk and left atrium in dogs. Thorax 1972;27:415-9.

43. Dark JH, Patterson GA, Al-Jilaihawi AN, et al. Experimental en bloc double-lung transplantation. Ann Thorac Surg 1986;42:394-8.

44. Patterson GA, Cooper JD, Dark JH, et al. Experimental and clinical double lung transplantation. J Thorac Cardiovasc Surg 1988;95:70-4.

45. Patterson GA, Todd TR, Cooper JD, et al. Airway complications after double lung transplantation. J Thorac Cardiovasc Surg 1990;99:14-20.

46. Schreinemakers HH, Weder W, Miyoshi S, et al. Direct revascularization of bronchial arteries for lung transplantation: an anatomical study. Ann Thorac Surg 1990;49:44-53.

47. Pettersson G, Arendrup H, Mortensen SA, et al. Early experience of double-lung transplantation with bronchial artery revascularization using mammary artery. Eur J Cardiothorac Surg 1994;8:520-4.

48. Couraud L, Baudet E, Nashef SA, et al. Lung transplantation with bronchial revascularization. Surgical anatomy, operative technique and early results. Eur J Cardiothorac Surg 1992;6:490-5.

49. Tong MZ, Johnston DR, Pettersson GB. The role of bronchial artery revascularization in lung transplantation. Thorac Surg Clin 2015;25:77-85.

50. Noirclerc MJ, Metras D, Vaillant A, et al. Bilateral bronchial anastomosis in double lung and heart-lung transplantations. Eur J Cardiothorac Surg 1990;4:314-7.

51. Pasque MK, Cooper JD, Kaiser LR, et al. Improved results for bilateral lung transplantation: rationale and initial experience. Ann Thorac Surg 1990;49:785-91.

52. Kaiser LR, Pasque MK, Trulock EP, et al. Bilateral sequential lung transplantation: the procedure of choice for double-lung replacement. Ann Thorac Surg 1991;52:438-45.

53. Bisson A, Bonette P. A new technique for double lung transplantation. "Bilateral single lung" transplantation. J Thorac Cardiovasc Surg 1992;103:40-6.

54. Taghavi S, Birsan T, Pereszlenyi A, et al. Bilateral lung transplantation via two sequential anterolateral thoracotomies. Eur J Cardiothorac Surg 1999;15:658-62.

55. Venuta F, Rendina EA, De Giacomo T, et al. Bilateral

sequential lung transplantation without sternal division. Eur J Cardiothorac Surg 2003;23:894-7.

56. Mal H, Andreassian B, Pamela F, et al. Unilateral lung transplantation in end-stage pulmonary emphysema. Am Rev Respir Dis 1989;140:797-802.

57. Mal H, Andreassian B, Pariente R. Single lung transplantation in hyperinflated patients. Chest 1990;97:110S-111S.

58. Sleiman C, Mal H, Andreassian B, et al. Single lung transplantation in pulmonary emphysema. N Engl J Med 1990;323:551-2.

59. Klepetko W, Grimm M, Laufer G, et al. One and one-half year experience with unilateral and bilateral lung transplantation. J Card Surg 1992;7:126-33.

60. Van Raemdonck D, Verleden G, Coosemans W, et al. Isolated lung transplantation; initial experience at the University Hospitals Leuven. Leuven Lung Transplant Group. Acta Chir Belg 1994;94:245-57.

61. Moser KM, Daily PO, Peterson K, et Al. Thromboendarterectomy for chronic, major vessel thromboembolic pulmonary hypertension: immediate and long term results. Ann Intern Med 1987;107:560-5.

62. Hsieh CM, Mishkel G, Radowski H, et al. Production and reversibility of right ventricular hypertrophy and right heart failure in dogs. Ann Thorac Surg 1992;54:104-10.

63. Pasque MK, Kaiser LR, Dresler CM, et al. Single lung transplantation for pulmonary hypertension. J Thorac Cardiovasc Surg 1992;103:475-81; discussion 481-2.

64. Pasque MK, Trulock EP, Cooper JD, et al. Single lung transplantation for pulmonary hypertension. Single institution experience in 34 patients. Circulation 1995;92:2252-8.

65. Birsan T, Zuckermann A, Artemiou O, et al. Functional results and long-term outcome after bilateral lung transplantation for pulmonary hypertension. Wien Klin Wochenschr 1998;110:45-52.

66. Marczin N, Royston D, Yacoub M. Pro: lung transplantation should be routinely performed with cardiopulmonary bypass. J Cardiothorac Vasc Anesth 2000;14:739-45.

67. McRae K. Con: lung transplantation should not be routinely performed with cardiopulmonary bypass. J Cardiothorac Vasc Anesth 2000;14:746-50.

68. Pereszlenyi A, Lang G, Steltzer H, et al. Bilateral lung transplantation with intra- and postoperatively prolonged ECMO support in patients with pulmonary hypertension. Eur J Cardiothorac Surg 2002;21:858-63.

69. Ius F, Kuehn C, Tudorache I, et al. Lung transplantation

on cardiopulmonary support: venoarterial extracorporeal membrane oxygenation outperformed cardiopulmonary bypass. J Thorac Cardiovasc Surg 2012;144:1510-6.

70. Biscotti M, Yang J, Sonett J, et al. Comparison of extracorporeal membrane oxygenation versus cardiopulmonary bypass for lung transplantation. J Thorac Cardiovasc Surg 2014;148:2410-5.

71. Bermudez CA, Shiose A, Esper SA, et al. Outcomes of intraoperative venoarterial extracorporeal membrane oxygenation versus cardiopulmonary bypass during lung transplantation. Ann Thorac Surg 2014;98:1936-42; discussion 1942-3.

72. Machuca TN, Collaud S, Mercier O, et al. Outcomes of intraoperative extracorporeal membrane oxygenation versus cardiopulmonary bypass for lung transplantation. J Thorac Cardiovasc Surg 2015;149:1152-7.

73. Christie JD, Edwards LB, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: 27th official adult lung and heart – lung transplant report--2010. J Heart Lung Transplant 2010;29:1104-18.

74. Schiavon M, Falcoz PE, Santelmo N, at al. Does the use of extended criteria donors influence early and long term results of lung transplantation? Interact Cardiovasc Thorac Surg 2012;14:183-7.

75. Meers C, Van Raemdonck D, Verleden GM, et al. The number of lung transplants can be safely doubled using extended criteria donors; a single-center review. Transpl Int 2010;23:628-35.

76. Somers J, Ruttens D, Verleden SE, et al. A decade of extended-criteria lung donors in a single center: was it justified? Transpl Int 2015;28:170-9.

77. Starnes VA, Lewiston NJ, Luikart H, et al. Current trends in lung transplantation: lobar transplantation and expanded use of single lungs. J Thorac Cardiovasc Surg 1992;104:1060-5; discussion 1065-6.

78. Starnes VA, Barr ML, Cohen RG. Lobar transplantation: indications, technique and outcome. J Thorac Cardiovasc Surg 1994;108:403-10.

79. Starnes VA, Barr ML, Cohen RG, et al. Living – donor lobar transplantation experience: intermediate results. J Thorac Cardiovasc Surg 1996;112:1284-90.

80. Starnes VA, Barr ML, Schenkel FA, et al. Experience with living – donor lobar lung transplantation for indications other than cystic fibrosis. J Thorac Cardiovasc Surg 1997;114:917-21.

81. Date H, Aoe M, Nagahiro I, et al. Living – donor lobar lung transplantation for various lung diseases. J Thorac

Cardiovasc Surg 2003;126:476-81.

82. Date H, Aoe M, Sano Y, et al. Improved survival after living – donor lobar transplantation. J Thorac Cardiovasc Surg 2004;128:933-40.

83. Camargo SM, Camargo JJP, Schio SM, et Al. Complications related to lobectomy in living lobar lung transplant donors. J Bras Pneumol 2008;34:256-63.

84. Chen QK, Jiang GN, Ding JA, et al. First successful bilateral living – donor lobar lung transplantation in China. Chin Med J 2010;123:1477-78.

85. Date H, Sato M, Aoyama A, et al. Living donor lobar lung transplantation provides similar survival to cadaveric lung transplantation even for very ill patients. Eur J Cardiothorac Surg 2015;47:967-72.

86. A definition of irreversible coma: report of the Ad Hoc Committee of the Harvard Medical School to Examine the Definition of Brain Death. JAMA 1968;205:337-40.

87. Kootstra G, Kievit J, Nederstigt A. Organ donors: heartbeating and non-heart beating. World J Surg 2002; 26:181-4.

88. Kootstra G, Daemen JH, Oomen AP. Categories of non-heart-beating donors. Transplant Proc 1995;27:2893-4.

89. Egan T, M, Lambert CJ, Reddick R, et al. A strategy to increase the donor pool: use of cadaver lungs for transplantation. Ann Thorac Surg 1991;52:1113-20.

90. Van Raemdonck DE, Rega FR, Neyrinck AP, et al. Non-heart-beating donors. Semin Thorac Cardiovasc Surg. 2004;16:309-21.

91. Love RB, Stringham JC, Chomiak PN, et al. Successful lung transplantation using a non-heart-beating donor. J Heart Lung Transplant 1995;14:S88.

92. Steen S, Sjöberg T, Pierre L, et al. Transplantation of lungs from a non-heart-beating donor. Lancet 2001;357:825-9.

93. Ruttens D, Martens A, Ordies S, et al. Short- and Long-Term Outcome After Lung Transplantation From Circulatory-Dead Donors: A Single-Center Experience. Transplantation 2017;101:2691-4.

94. Krutsinger D, Reed RM, Blevins A, et al. Lung transplantation from donation after cardiocirculatory death: a systematic review and meta-analysis. J Heart Lung Transplant 2015;34:675-84.

95. Cypel M, Levvey B, Van Raemdonck D, et al. International Society for Heart and Lung Transplantation Donation After Circulatory Death Registry Report. J Heart Lung Transplant 2015;34:1278-82.

96. Nuñez JR, Varela A, del Rio F, et al. Bipulmonary transplants with lungs obtained from two non-heart-beating donors who died out of hospital. J Thorac

Cardiovasc Surg 2004;127:297-9.

97. Gomez-de-Antonio D, Campo-Cañaveral JL, Crowley S, et al. Clinical lung transplantation from uncontrolled non-heart-beating donors revisited. J Heart Lung Transplant 2012;31:349-53.

98. Suzuki Y, Tiwari JL, Lee J, et al. Should we reconsider lung transplantation through uncontrolled donation after circulatory death? Am J Transplant 2014;14:966-71.

99. Valenza F, Citerio G, Palleschi A, et al. Successful Transplantation of Lungs From an Uncontrolled Donor After Circulatory Death Preserved In Situ by Alveolar Recruitment Maneuvers and Assessed by Ex Vivo Lung Perfusion. Am J Transplant 2016;16:1312-8.

100. Steen S, Liao Q, Wierup PN, et al. Transplantation of lungs from non-heart-beating donors after functional assessment ex vivo. Ann Thorac Surg 2003;76:244-52.

101. Van Raemdonck D, Neyrinck A, Cypel M, et al. Ex-vivo lung perfusion. Transpl Int 2015;28:643-56.

102. Jirsch DW, Fisk RL, Couves CM. Ex vivo evaluation of stored lungs. Ann Thorac Surg 1970;10:163-8.

103. Sanchez PG, Bittle GJ, Burdorf L, et al. State of art: clinical ex-vivo lung perfusion: rationale, current status, and future directions. J Heart Lung Transplant 2012;31:339-48.

104. Lindstedt S, Eyjolfsson A, Koul B, et al. How to recondition ex vivo initially rejected donor lungs for clinical transplantation: clinical experience from the Lund university hospital. J Transplant 2011;2011:754383.

105. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. N Engl J Med 2011;364:1431-40.

106. Machuca TN, Mercier O, Collaud S, et al. Lung transplantation with donation after circulatory determination of death donors and the impact of ex vivo lung perfusion. Am J Transplant 2015;15:993-1002.

107. Wigfield CH, Cypel M, Yeung J, et al. Successful emergent lung transplantation after ex vivo perfusion optimization and transportation of donor lungs. Am J Transplant 2012;12:2838-44.

108. Machuca TN, Hsin MK, Ott HC, et al. Injury-specific ex vivo treatment of the donor lung: pulmonary thrombolysis followed by successful lung transplantation. Am J Respir Crit Care Med 2013;188:878-80.

109. Inci I, Yamada Y, Hillinger S, et al. Successful lung transplantation after donor lung reconditioning with urokinase in an ex-vivo lung perfusion system. Ann Thorac Surg 2014;98:1837-8.

110. Cypel M, Keshavjee S. Extending the donor pool: rehabilitation of poor organs. Thorac Surg Clin 2015;25:27-33.

111. Ordies S, Neyrinck A, Van Raemdonck D. Can we make recovered donor lungs look brand-new again? Eur J Cardiothorac Surg 2017;52:178-9.

112. Warnecke G, Van Raemdonck D, Kukreja J, et al. Mid and long-term clinical results of OCS Lung Inspire International trial. J Heart Lung Transplant 2016;35:S15-16.

Lung transplantation: indications and contraindications

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Abstract: The selection of appropriate recipients for lung transplantation is an evolving discipline. As experience with the procedure has developed over the last decades, the identification of transplant candidates has also changed as transplant centers strive to safely provide the therapy to as many patients possible. The International Society for Heart and Lung Transplantation (ISHLT) has developed three editions of recipient selection guidelines. Published in 1998, 2006, and 2015, these guidelines represented the best information relevant to the appropriate selection of lung transplant candidates. A discussion of areas supported by the most robust scientific data will be undertaken, but in many aspects of recipient selection, there is a paucity of data upon which to rely. Therefore, it is ultimately the prerogative and responsibility of individual centers to determine, after carefully weighing the best evidence available, whether a patient is deemed a suitable candidate at a specific program. All possible indications and contraindications for transplantation will be reviewed with attention also given to the appropriate timing of referral and listing of patients with advanced lung disease to a transplant center.

Keywords: Interstitial lung disease (ILD); chronic obstructive pulmonary disease (COPD); pulmonary arterial hypertension; cystic fibrosis (CF)

"Because donated organs are a severely limited resource, the best potential recipients should be identified. The probability of a good outcome must be highly emphasized to achieve the maximum benefit for all transplants."

OPTN/UNOS Ethics Committee General Considerations in Assessment for Transplant Candidacy. HRSA; 2010.

This chapter will discuss lung transplant indications and contraindications, representing a consensus of expert opinion developed over the years. The International Society for Heart and Lung Transplantation (ISHLT) has developed three editions of recipient selection guidelines. Published in 1998, 2006, and 2015 (1-3), these guidelines represented the best information relevant to the appropriate selection of lung transplant candidates. Although an effort will be made to include a discussion of areas supported by robust scientific data, as in many aspects of recipient selection, there is a paucity of data upon which to rely. Therefore, it is ultimately the prerogative and responsibility of individual centers to determine, after carefully weighing the best evidence available, whether a patient is ultimately deemed a suitable candidate at a specific program.

Recipient considerations

Lung transplantation should be considered for adults with advanced lung disease who meet the following general criteria:

(I) High (>50%) risk of death due to lung disease within 2 years if lung transplantation is not performed;

(II) High (>80%) likelihood of surviving at least 90 days after lung transplantation;

(III) High (>80%) likelihood of 5-year post-transplant survival from a general medical perspective provided there is adequate graft function.

Contraindications

Lung transplantation is a complex therapy with a significant

risk of perioperative morbidity and mortality. Therefore, it is prudent to consider all contraindications and comorbidities. The following lists are not intended to cover all possible clinical scenarios, but do highlight common areas of concern.

Absolute contraindications

❖ Lung transplantation should not be offered to adults with a recent history of malignancy. A 2-year disease-free interval combined with a low predicted risk of recurrence after lung transplantation may be reasonable, for instance in skin cancers other than melanoma that have been treated appropriately. However, a 5-year disease-free interval should be demonstrated in most cases, particularly for those with a history of hematologic malignancy, sarcoma, melanoma, or cancers of the breast, bladder, or kidney. Unfortunately, for some patients with a history of cancer, the risk of recurrence may remain too high to proceed with lung transplantation even after a 5-year disease-free interval;

❖ Poorly controlled significant dysfunction of another major organ system (e.g., heart, liver, kidney or brain) unless a multi-organ transplant is being considered;

❖ Uncorrected coronary artery disease with end-organ ischemia or dysfunction and/or coronary artery disease not amenable to revascularization;

❖ An unstable medical condition, including but not limited to acute sepsis, myocardial infarction, and liver failure;

❖ Uncorrectable bleeding disorder;

❖ Poorly controlled infection with a virulent and/or resistant microbes;

❖ Evidence of active *Mycobacterium tuberculosis* infection;

❖ A chest wall or spinal deformity expected to cause severe restriction after transplantation;

❖ Class II or III obesity (BMI $\geq$35.0 kg/m^2);

❖ Current non-adherence to medical therapy or a history of repeated or prolonged episodes of non-adherence to medical therapy that are perceived to increase the risk of non-adherence after transplantation;

❖ Psychiatric or psychological issues likely rendering the patient unable to comply with a complicated medical regimen;

❖ Inadequate social support system;

❖ Functionally limited with inability to participate in a rehabilitation program;

❖ A history of illicit substance abuse or dependence (e.g., alcohol, tobacco, marijuana, or other illicit substances). Convincing evidence of risk reduction behaviors (such as participation in therapy for substance abuse and/or dependence) should be demonstrated before lung transplantation is considered. Periodic blood and urine testing can be utilized to verify abstinence.

Relative contraindications

❖ Age over 65 years in association with low physiological reserve and/or other relative contraindications. Although there cannot be endorsement of an upper age limit as an absolute contraindication, adults older than 75 years of age are less likely to be candidates for lung transplantation. Although age alone should not exclude a patient from receiving a lung transplant, increasing age often is associated with comorbid conditions that are either absolute or relative contraindications;

❖ Class I obesity (BMI 30.0 to 34.9 kg/m^2), particularly truncal (central) obesity;

❖ Significant malnutrition;

❖ Significant osteoporosis;

❖ Extensive prior chest surgery with lung resection;

❖ Mechanical ventilation and/or extracorporeal life support (ECLS). However, carefully selected candidates without other acute or chronic organ dysfunction may be successfully transplanted;

❖ Colonization with resistant or highly virulent pathogens;

❖ For candidates infected with hepatitis B and/or C, lung transplant can be considered in patients without significant clinical, radiological, or biochemical signs of cirrhosis or portal hypertension and who are stable on appropriate therapy. Lung transplantation in hepatitis B and/or C candidates should be performed in centers with experienced hepatology units;

❖ For patients infected with human immunodeficiency virus (HIV), lung transplant can be considered in those with controlled disease with undetectable HIV-RNA, and adherent with anti-retroviral therapy (cART). Lung transplantation in HIV positive candidates should be performed in centers with expertise in the care of HIV positive patients;

❖ Infection with *Burkholderia cenocepacia*, *Burkholderia gladioli*, and multi-drug resistant *Mycobacterium abscessus* if the infection is sufficiently treated

preoperatively and there is a reasonable expectation for adequate control postoperatively. In order for patients with these infections to be considered suitable transplant candidates, patients should be evaluated by centers with significant experience managing these infections in the transplant setting, and patients should be aware of the increased risk of transplant due to these infections;

❖ Coronary artery disease burden sufficient to put the candidate at risk for end-organ disease after lung transplantation. The preoperative evaluation, type of coronary stent used, and extent of coronary artery disease considered acceptable varies among transplant centers;

❖ Extrapulmonary conditions that have not resulted in significant organ damage, such as diabetes mellitus, systemic hypertension, epilepsy, central venous obstruction, peptic ulcer disease, or gastroesophageal reflux should be well-controlled before transplantation.

Special surgical considerations

Previous surgery

Recommendations

❖ Previous surgery is not a contraindication to lung transplantation;

❖ Previous pleurodesis can present operative challenges but is not a contraindication;

❖ Pneumothorax in a patient who may become a future transplant recipient should be given the best immediate management. The choice of intervention is unlikely to affect future acceptance for transplantation;

❖ Higher rates of bleeding, re-exploration and renal dysfunction are to be expected in patients with previous chest procedures. This may be exacerbated by longer cardiopulmonary bypass times;

❖ In otherwise well selected patients, medium and long-term outcome is not affected by previous chest procedures;

❖ Conversely, older patients (>65) with other co-morbidities have poorer outcomes, and the previous intrapleural procedure should be taken into account during selection.

Some patients referred for lung transplantation will have undergone previous chest surgery. If one includes prior chest tube insertion, the percentage of referred patients

may be up to 40% (3,4) or for up to 90% in conditions such as lymphangioleiomyomatosis (LAM) (5). Surgery may be coincidental, for instance previous coronary artery bypass grafting (CABG), but usually related as a diagnostic or therapeutic step in pre-transplant management. Examples of the latter range from simple video-assisted thoracoscopic (VATS) biopsy in interstitial disease to previous lung volume reduction surgery (LVRS). Conditions associated with recurrent pneumothorax, such as cystic fibrosis (CF) or LAM may have required pleurodesis, previous lung resection, or pneumonectomy.

The evidence for any effect of previous interventions is entirely based on retrospective institutional or local registry reports, and so is prone to publication bias. There have been small series (14 and 18 patients) (5,6) describing successful lung transplant after chest surgery. The largest recent experience (7) described 238 patients, although 115 merely had earlier chest drain insertion. A number of accounts concentrate on conditions such as LAM (5) or CF (8) where pneumothorax is a disease specific pre-transplant complication.

Some broad conclusions can be taken from the published literature. Any previous surgery, but particularly pleurodesis (surgical or chemical), is associated with higher blood loss and early post-operative morbidity such as renal dysfunction and primary graft dysfunction. There is also a higher incidence of phrenic nerve damage, chylothorax and re-exploration. Not surprisingly, where multivariate analysis can be applied (4), the combinations of age >65 years, pulmonary hypertension, transfusion >20 units and prolonged cardiopulmonary bypass are all predictors of early death. Previous cardiac surgery appears to have little specific effect, but reported experience is very small.

The specific issue of previous LVRS is examined in several papers. Early experience indicated that LVRS had no effect (9), but a more recent account (10), where 25 out of 177 patients transplanted for chronic obstructive pulmonary disease (COPD) had undergone previous LVRS had poorer outcomes. There were the expected higher rates of bleeding and early morbidity, but also a significantly worse early graft function, and poorer results in older, frailer patients.

Mechanical bridges to transplant

ECLS recommended:
❖ Young age;
❖ Absence of multiple-organ dysfunction;
❖ Good potential for rehabilitation.

ECLS not recommended:

- ❖ Septic shock;
- ❖ Multi-organ dysfunction;
- ❖ Severe arterial occlusive disease;
- ❖ Heparin-induced thrombocytopenia;
- ❖ Prior prolonged mechanical ventilation;
- ❖ Advanced age;
- ❖ Obesity.

"Bridge to lung transplantation" refers to strategies to manage with artificial support the acutely decompensating patient until a suitable organ is available (11). Ideally, bridge to lung transplantation should be applied with the intent to prolong both the pre-transplant life expectancy of patients increasing the chances to receive a lung transplant and improving the likelihood of a successful post-transplant outcome by improving pre-transplant clinical stability. It is also preferable that patients bridged to transplant in this way have already been fully evaluated by the transplant team and all medical and psychosocial risk factors identified prior to initiating bridge therapy. Less favorable outcomes are generally seen in those patients who present *de novo* with respiratory failure and are placed on a mechanical support system without the benefit of having the transplant team and the patient having fully considered transplant as a therapeutic option.

Mechanical ventilation today has been the most commonly used bridging strategy to lung transplant (12-16), but ventilated patients are particularly susceptible to ventilator-induced lung injury and ventilator-associated pneumonia and requires patients to be bed-bound and often sedated, which reduces their ability to undergo adequate physiotherapy. This can lead to severe deconditioning and may compromise their suitability for transplantation. Thus, while often successful, mechanical ventilation is far from the "ideal bridge" to lung transplant.

Since the beginning of the lung transplant era, ECLS has been recognized as a potential bridge to lung transplant for patients with respiratory failure. However, the initial clinical experience in the 1980's and 1990's was discouraging with a high mortality rate and a high incidence of complications associated with the application of ECLS (17). In recent years, substantial improvements in the ECLS technology have led to renewed enthusiasm for ECLS as a bridge to lung transplant. Current ECLS devices can provide different modes and configurations of support with the appropriate level of pulmonary (and cardiac) support for each patient's physiological need with significantly less morbidity and complications (11-13,17,18).

In the modern era of ECLS, several recently published case series have shown that the post-transplant mortality rate of selected patients bridged to transplant with ECLS is comparable to that of patients transplanted without pre-transplant ECLS (17-23). Despite these promising results, the application of ECLS as bridge to transplant remains controversial. In addition to the historically poor outcomes, bridging patients to transplant with ECLS is associated with substantial resource utilization both in the pre and post-transplant phase and is associated with important complications (bleeding, vascular access problems, infection). However, it should be noted that the transplant benefit is likely higher in this patient group, given the high pre-transplant mortality associated with the need for this level of support. Regardless, it is well accepted by centers utilizing ECLS that post-transplant mortality increases in relation to time on ECLS pre-transplant and caution should be exercised in transplanting candidates who have prolonged need for ECLS.

Recently, newer ECLS systems have maintained patient stability with fewer complications. As a bridge to lung transplant, ECLS is being progressively used as an alternative to mechanical ventilation to avoid the injurious side effects of mechanical ventilation, rather than as a rescue treatment for patients' refractory to mechanical ventilation. Fuehner and colleagues (23) have published one of the first reports showing that in patients bridged to lung transplant with ECLS the post-transplant survival rate was higher than in historical control patients bridged with invasive mechanical ventilation (80% *vs.* 50%, P=0.02). In this study ECLS was applied in awake non-intubated patients who were allowed to ambulate while on ECLS and receive active physical therapy (23). In a recent analysis of the UNOS data, 1-year survival in patients bridged to transplant using ECLS substantially improved from 30% in 2005 to 75% in 2010 at which time survival was superior to those who were transplanted off a ventilator.

Indications and contraindications to ECLS as a bridge to transplant cannot be firmly established as only relatively small case series have been published to date. However, recommendations for the use of ECLS have been published (11,13).

ECLS is effective in supporting potential recipients with advanced respiratory failure and to improve patients' clinical stability, which should ultimately improve post-transplant outcomes. Clinical advancements in this field are needed, as the mortality rate of patients on the lung transplant waiting list is still in the range of 20% (24). Bridging to

transplant using ECLS requires ongoing assessment of the potential recipient for candidacy as frequently neurologic events, organ failure and infectious complications preclude candidacy for transplantation.

Disease-specific indications and considerations

IPF

Indications

Interstitial lung disease (ILD), and specifically idiopathic pulmonary fibrosis (IPF), carries the worst prognosis among the common disease indications for lung transplantation. Worldwide changes in donor lung allocation, including the Lung Allocation Score (LAS) in the USA and in Eurotransplant, have dramatically increased lung transplant rates for candidates with ILD. Despite this, waiting list mortality remains high. In phase 3 trials of patients with IPF, pirfenidone was shown to reduce disease progression, as reflected by lung function, exercise tolerance, and survival (25). In the most recent American Thoracic Society (ATS) consensus document, transplantation and supplemental oxygen were the only treatments strongly recommended for patients with IPF, and a transplant discussion was recommended at the time of diagnosis (26). The evidence reviewed here will focus on IPF as the most common and life-threatening subtype of ILD, while recognizing that fibrosing nonspecific interstitial pneumonia (NSIP) and other types of progressive ILD refractory to treatment may carry a similar prognosis. Prognosis in IPF is generally poor; retrospective cohort studies indicate a median survival of 2–3 years from diagnosis, and only 20–30% patients survive more than 5 years after diagnosis (26,27). This underscores the importance of early referral of IPF patients so that listing and transplantation can be achieved rapidly in the setting of an unexpected decline (28).

Prognostic factors in IPF have recently been reviewed in detail (26) and consistent clinical predictors of worse survival include older age, dyspnea, low or declining pulmonary function (28-31), pulmonary hypertension, concomitant emphysema, extensive radiographic involvement, low exercise capacity or exertional desaturation (28,32), and usual interstitial pneumonitis (UIP) on histopathology. Clinical prediction models such as the clinical, radiologic, and physiologic (CRP) score have not been widely used in practice (33). Also, du Bois and colleagues assessed numerous risk factors in a large cohort of IPF patients and developed a practical 4-item risk scoring system, which

includes age, respiratory hospitalization, percent predicted FVC, and 24-week change in forced vital capacity (FVC) (30). If validated, particularly in IPF patients who are potential lung transplant candidates, this model could be a useful aid in referral and listing decisions.

Special considerations

ILD severe enough to warrant consideration of lung transplantation may be associated with collagen vascular diseases such as scleroderma and rheumatoid arthritis. Data regarding specific predictors of prognosis in this setting are limited (34,35). If the lung disease has not responded to appropriate treatment and there are no extrapulmonary contraindications to transplantation, it is reasonable to use similar guidelines to those proposed for idiopathic ILD.

CF

Indications

Transplantation should be considered for suitable CF patients who have less than a 50% 2-year predicted survival and whom have functional limitations classified as New York Heart class III or IV. Predicting survival using objective data, however, has been difficult with no single factor sufficiently predictive of poor survival in CF patients. Much of the data applies to the general CF population rather than the population that meets other criteria for transplantation and the CF transplant candidate data comes from relatively small cohorts. A measurement of lung function over time to assess disease progression has been the most useful predictor (36). The FEV_1 has been the most frequently used variable in assessing early mortality. In 1992, Kerem *et al.* reported that an FEV_1 less than 30% of predicted was associated with a 2-year mortality rate of approximately 40% in men and 55% in women (37).

Mayer-Hamblett and colleagues utilized the Cystic Fibrosis Foundation registry to develop a model identifying the best clinical predictors of mortality in the CF patients. They found that age, height, FEV_1, respiratory microbiology, number of hospitalizations, and the number of home intravenous antibiotic courses were significant predictors of 2-year mortality, but their multivariate logistic regression model was not a better predictor of early mortality than the FEV_1 alone (38). Another study evaluated CF patients referred for transplantation at four lung transplant centers. Using a univariate analysis, the authors reported a relationship between early mortality and

an FEV_1 less than 30% of predicted and an elevated $PaCO_2$ >50 mmHg (6.6 kPa). They also noted the need for and the use of nutritional supplements as an indicator of increased early mortality. Those patients who had an FEV_1 less than 30% of predicted had an increased early mortality only when their PCO_2 was greater than 50 mmHg (39). Milla and Warwick in their single-center study also found that the rate of decline was a better predictor of early mortality than the FEV_1 alone (40). Using the Cystic Fibrosis Foundation database, Liou and colleagues developed a 5-year survival model (41). The authors evaluated the impact of various variables on survival and correlated it with a change in the FEV_1 percent predicted. They found that the female sex, diabetes mellitus, *Burkholderia cepacia* infection, and the number of exacerbations negatively impacted the survival of the CF patient whereas FEV_1 percent predicted alone was not a sufficient predictor of early mortality.

Other preoperative characteristics that impact survival following lung transplantation are exercise tolerance and pulmonary hypertension. A 6-minute walk distance less than 400 meters and pulmonary hypertension have been associated with poor outcomes (42-45). The development of a pneumothorax and the presence of non-tuberculous mycobacterial (NTM) disease (in particular *M. abscessus*) also increase declines in lung function and or mortality in those with advanced lung disease (46,47).

Specific considerations

Non-tuberculous mycobacteria

There has been an observed increase in incidence of patients with CF culturing NTM (45). The following recommendations are made, though it is recognized that this is a subject where the evidence is predominantly based on case series (48,49):

(I)   All patients with CF who are referred for transplantation should be evaluated for NTM pulmonary disease;
(II) Patients with NTM disease who are being evaluated for transplantation should have the organism confirmed according to microbiology guidelines and commence treatment before transplant listing;
(III) Treatment should be by, or in collaboration with, a physician experienced in the treatment of such patients;
(IV) Patients with progressive pulmonary or extrapulmonary disease due to NTM despite

optimal therapy or an inability to tolerate optimal therapy is a contraindication for transplant listing.

Burkholderia cepacia complex

CF patients who are infected with *B. cepacia* have been shown to have a more rapid progression of respiratory disease associated with a more rapid fall in FEV_1. *Burkholderia cepacia* complex patients also have a less favorable outcome post transplantation, though the majority of the increased risk has been shown to be confined to those patients infected with the species *Burkholderia cenocepacia* (50-52).

The following recommendations are made:
❖ All patients with CF referred for transplantation should be evaluated for the presence of B. cepacian;
❖ Patients with species other than *B. cenocepacia* do not constitute an increased risk for mortality after transplantation and can be listed providing other criteria are met;
❖ Patients with *B. cenocepacia* have an increased risk of mortality due to recurrent disease after transplantation. It is recommended that centers continuing to accept such patients should have an active research program assessing novel approaches to prevent and control recurrent disease and should be experienced in management of these patients. Further, a full discussion with the patients of the increased risk associated with these infections should occur.

Indications

With 40% of all lung transplantations performed worldwide, COPD (non-A1ATD and A1ATD) is the most common indication (53). The clinical course of COPD is typically very protracted, and even at an advanced stage, short—and intermediate—survival is better than the other commonly transplanted diseases. Apart from survival, in patients with COPD, the most important clinical feature is a decline in the quality of life. As a result, considering the prevalence of end-stage COPD and the continuing donor organ shortage, it remains challenging to decide when to list COPD patients and whether quality of life issues should also be taken into account when making that decision.

In a recent study including 609 patients with severe emphysema randomized to the medical therapy arm of the National Emphysema Treatment Trial (NETT), Martinez *et al.* identified the following factors, which were associated with increased mortality in a multivariate

analysis: increasing age, oxygen utilization, lower total lung capacity and higher residual volume (%predicted), lower maximal cardiopulmonary exercise testing workload, greater proportion of emphysema in the lower lung zone versus the upper lung zone and lower upper-to-lower-lung perfusion ratio. Also, the modified BODE score, which is a composite score of body mass index (B), %predicted FEV_1 (airway obstruction, O), dyspnea (D) and exercise capacity (E) was associated with a higher mortality (54). In some studies, the original BODE score, developed by Celli *et al.* (55) assigned a score from 0 to 10, with a higher score indicating more severe disease and a worse survival (a BODE score of 7–10 was associated with a mortality of 80% at 4 years, whereas a score of 5–6 conferred a mortality of 60% at 4 years) and proved to be a better indicator of survival than the spirometric staging system (56). Either the original or modified BODE can be used, depending on local center preference and expertise.

The presence of 3 or more exacerbations in a 1-year period negatively impacts survival in COPD patients (57). Moreover, the mortality risk is independent of the severity of the disease as measured by the BODE index (58). COPD patients with acute hypercapnic respiratory failure have an in-hospital mortality of >10% and subjects who survived the hospital admission have a 43% and 49% mortality rate at 1 and 2 years post admission (59).

The role of the BODE score and its impact on lung transplantation survival for COPD has recently been evaluated by Lahzami *et al.* who showed that the majority of COPD patients had survival benefit from lung transplantation regardless of their pre-transplant BODE score, although a global survival benefit was only seen in patients with a BODE score of 7 or more (60), suggesting that this is the appropriate population to transplant. Patients with a BODE index of 5–6, while not expected to derive a survival benefit, experienced similar quality of life benefits from transplant to patients with a BODE index of 7–10. Although lung transplant candidates with COPD are different compared to the original COPD population as assessed in the BODE index paper by Celli *et al.* (younger age and non-smoking), it does not prevent the BODE index from being useful in the assessment of COPD candidates for lung transplantation (61).

Special considerations

A specific issue to the COPD population is the impact of bronchoscopic [bronchoscopic lung volume reduction (BLVR)] or LVRS on listing for lung transplantation. In certain patients (FEV_1 <25%, but >20%, DL_{CO} >20% and heterogeneous emphysema distribution on CT scan) LVRS may be offered first, reserving transplantation for those patients failing to improve with LVRS or in those experiencing a lung function decline after a period of sustained improvement. Successful LVRS with improvement in functional and nutritional status can improve the patient's suitability as a transplant candidate (9,62).

Pulmonary hypertension

Indications

The timing of referral for transplant for pulmonary vascular disease remains difficult. The development of targeted medical therapy has led to a marked change in the timing for referral and listing for patients with idiopathic pulmonary arterial hypertension (IPAH) or pulmonary hypertension from other causes. Medical therapies including the prostanoids, endothelin receptor antagonists and phosphodiesterase inhibitors have proven efficacy in the management of IPAH patients and, as such, the majority of patients who would have been listed for transplant in the pre-prostanoid era may not require transplant listing while awaiting clinical response to medical therapy (63-65). Because of the generally good response to medical therapy, transplant centers still vary considerably in referral, listing and transplantation of IPAH patients. However, in patients who are deteriorating rapidly, transplant bridging strategies are an option but a more difficult one in this patient group.

Equations to predict waitlist mortality in patients with IPAH are under development. One such Registry with a published equation, the U.S. Registry to Evaluate Early and Long-term PAH disease Management (REVEAL), identified the following factors to be associated with increased mortality: functional class IV, male gender with age >60 years old, increased pulmonary vascular resistance (PVR), PAH associated with portal hypertension or a family history of PAH (66). Functional class III, increased mean right atrial pressure, decreased resting systolic blood pressure or an elevated heart rate, decreased six-minute walk distance, increased brain natriuretic peptide, renal insufficiency, PAH associated with connective tissue diseases, a decreased carbon monoxide diffusing capacity or the presence of a pericardial effusion were also associated with increased mortality. Despite criticism that this Registry did not reflect actual lung transplant waitlist populations, it provided insight into risk factors for mortality.

Special transplant circumstances

Lung retransplantation

Lung retransplantation accounts for a small percentage of lung transplants performed annually. However, its frequency has increased in recent years. This trend has been particularly true in North America and coincided with the introduction of the lung allocation scoring (LAS) system in 2005 in the United States. While many of these patients would previously have been too ill to survive prolonged wait times, the LAS system has allowed them priority access to available donor organs (67,68).

The criteria for candidate selection for lung retransplantation generally mirror those utilized for selection for initial lung transplantation. Important considerations include the presence of significant renal dysfunction, which, if present, increases the hazard ratio for mortality considerably among retransplantation candidates. The presence of additional comorbidities also increases risk by a multivariate analysis (69,70).

Retransplantation candidates may be considered for bilateral lung or single lung transplantation. If the initial transplant was a single lung transplant, consideration must be given to whether leaving the previous allograft *in situ* is desirable. The failed allograft may represent a source of ongoing immune stimulation, and its removal would offer intuitive advantages. Previous reports have also identified the retained allograft as a source of fatal infection in nearly one quarter of retransplantation recipients (71). These factors would suggest that failed allograft removal is advisable. Ipsilateral single lung retransplantation has been associated with a higher acute risk of death when compared to contralateral single lung retransplantation (69). However, these comparisons are somewhat confounded by factors such as the original indication and timing for retransplantation. Nonetheless, the most recent trend has been toward more frequent bilateral retransplantation. This may relate to a desire to remove failed allografts in an era when initial bilateral lung transplantation is increasingly more common.

Factors have been identified that influence short and long-term outcomes after lung retransplantation (72,73). Patients retransplanted for bronchiolitis obliterans syndrome (BOS) manifest better survival than those transplanted for primary graft dysfunction or airway complications. Generally, patients that are more than two years out from initial transplantation fare better than those retransplanted earlier. Those retransplanted for BOS in general demonstrate more rapidly declines in airflow than those transplanted for other indications. However, those retransplanted less than 2 years following initial transplantation also have an even greater risk of developing BOS (70).

It is generally accepted that those patients who were mechanically ventilated immediately prior to retransplantation have inferior survival outcomes. More recent analysis (73) have suggested that when patients retransplanted less than 30 days of initial transplantation are excluded, mechanical ventilation is not an independent risk factor for poor outcomes. However, in centers performing a high volume of retransplant operations, poorer outcomes have been observed in patients who are hospitalized, with or without the need for mechanical ventilation.

Survival after lung retransplantation may have improved over time but remains inferior to that seen after initial transplantation. In fact, for the individual patient, retransplantation should be analyzed as a time dependent survival risk factor. Consideration must also be given to ethical issues surrounding lung allocation to retransplantation candidates. Prioritization of younger patients in consideration for retransplantation is consistent with public preference. However, categorically placing older patients at a disadvantage is inappropriate.

Heart-lung transplant

Patients with end-stage lung and heart diseases not amenable to either isolated heart or lung transplant may be candidates for heart-lung transplantation. In most circumstances, patients with irreversible myocardial dysfunction or congenital defects with irreparable defects of the valves or chambers in conjunction with intrinsic lung disease or severe pulmonary artery hypertension are considered for heart-lung transplantation (74-76).

Pulmonary artery hypertension and elevated PVR should be considered as relative contraindications to isolated cardiac transplantation defined as a PVR is >5 Woods units, a PVR index is >6 or a transpulmonary pressure gradient (TPG) 16–20 mmHg. If the pulmonary artery systolic pressure (PAS) exceeds 60 mmHg in conjunction with any of the aforementioned 3 variables, the risk of right heart failure and early death is increased. If the PVR can be reduced to <2.5 with a vasodilator but the systolic blood pressure falls to <85 mmHg, the patient remains at high risk of right heart failure and mortality after isolated cardiac transplantation. Mechanical circulatory

support can improve these parameters and still make heart transplantation a possibility, obviating the need for heart lung transplantation.

In the clinical scenario of pulmonary hypertension and right ventricular failure, isolated double lung transplantation is associated with similar outcomes as seen in heart-lung transplantation (77). In the absence of objective assessment of infarcts or fibrotic changes of the right ventricle, heart-lung transplantation is usually not indicated. Exceptions may occur such as when the heart size occupies the majority of the thoracic cavity and would critically limit the available thoracic volume for the lung allografts.

In patients with intrinsic cardiac diseases such as coronary artery disease, valvular heart disease or septal defects (77), without intrinsic myocardial dysfunction, corrective cardiac surgery with concomitant lung transplant is preferable to heart-lung transplantation.

Patients with sarcoidosis involving both the heart and lungs may be best managed with heart-lung transplantation.

The timing of transplantation, particularly in patients with congenital heart disease, can be challenging. However, indices of right ventricular failure such as persistent class IV symptoms on maximal medical therapy, with cardiac index of less than 2 liters/min/m^2 and right arterial pressure exceeding 15 mmHg are indications to proceed with transplant listing. Certain anomalies such as pulmonary venous stenosis or pulmonary veno-occlusive disease (PVOD) in conjunction with the need to replace the heart respond poorly to medical management and often require earlier transplant listing.

Multi-organ transplant

There is an expanding pool of potential candidates with multisystem organ dysfunction who might benefit from simultaneous lung transplant and transplantation of another solid organ. Concurrent thoracic and abdominal transplantation was recently reviewed by Wolf *et al.* (78), who analyzed 122 simultaneous lung-liver transplants (typically for cystic fibrosis) and 41 lung-kidney transplants (typically for restrictive lung disease or pulmonary hypertension). The authors concluded such patients had high waiting list mortality at 34% and 35% respectively, although having reached transplantation, the simultaneous procedure conferred a significantly enhanced 5-year survival at 59% and 56%, respectively. These survival figures are actually higher than those of lung transplantation alone [50% at 5 years in the USA (P<0.01)], although less than

that of abdominal transplantation alone. This may reflect the expertise of the centers attempting such transplants. These pooled results are consistent with other small case series from the USA and Europe (79,80).

Combined lung and kidney transplant

The most common combination of thoracic and abdominal transplantation is kidney transplantation following lung transplantation. Cassuto and coworkers (81) have reviewed the UNOS deceased donor experience and noted 362 lung transplant recipients had been listed for kidney transplant at a mean of 6.5 years post-lung transplant. It is clear from this statistic that staged kidney transplants relatively soon after transplant are rare with most representing the failure of a second organ system due to the effects of calcineurin inhibitors.

When considering the overall survival benefit, kidney transplantation following lung transplantation was poorest of the solid organ combinations and related to the lung allograft with 80% dying with a functional kidney graft. Interestingly, a living-related kidney transplant effectively doubles the survival compared to a deceased donor with a longer wait time. Lonze *et al.* (82) subsequently produced a similar analysis, reinforcing the high waiting list mortality and need to consider living-related and extended donor criteria kidneys to optimize access to transplantable kidneys. Most lung transplant recipients with advanced kidney disease will not survive the wait time for a cadaveric kidney and the impact of the renal failure on lung function plays a significant component of the patient's respiratory decline.

Combined lung and liver transplant

The referral of lung transplant candidates with both advanced liver and lung disease is increasing. In some instances, the liver and lung disease are part of the same disease process, such as in CF and A1ATD, but in other patients, the disease process affecting each organ is separate. The information available regarding combined liver-lung transplant is derived from case series and the UNOS database, and the number of cases currently reported is small (less than 100) (79,80,83-87). Based on the information available, candidates for combined lung-liver transplant should meet lung disease specific criteria for lung transplant listing and have advanced liver disease as demonstrated by biopsy-proven cirrhosis and a portal gradient >10 mmHg. Combined liver lung transplant should not be considered

in those patients with albumin <2.0 g/dL, international normalized ratio (INR) >1.8, or the presence of severe ascites or encephalopathy. In some patients with less severe liver or lung disease, listing for a combined transplant may be appropriate if post-transplant organ dysfunction would be anticipated if the patient were to receive either single organ alone. In this situation, multiple factors may influence the decision regarding combined transplant or liver or lung transplant alone and include: anticipated wait time for the combined and single organ, anticipated level of liver or lung dysfunction after undergoing a single organ transplant, amount of bleeding expected in those with liver disease, rate of expected progression of the liver or lung disease after transplantation of the other organ, and presence of co-morbidities which could complicate the postoperative recovery of the combined transplant recipient.

Esophageal dysfunction/scleroderma

Lung transplantation for systemic sclerosis (SSc) remains controversial. Despite previous inclusion as an acceptable indication for transplant in the ISHLT guidelines for lung transplantation (1), due to concerns about esophageal dysmotility and gastroparesis increasing the risk of aspiration, many centers continue to consider SSc a contraindication. Two recent reports suggest that patients with SSc, even in the presence of esophageal disease, have similar 1 and 5-year survival rates to other ILD patients (88,89). Rates of acute rejection were increased in SSc patients in one report (88) and no different in the other (89). Importantly, incidence of BOS was similar between the two groups in both reports. Carefully selected SSc patients can undergo successful lung transplantation. Care to rule out intrinsic renal disease and measures to control esophageal dysmotility post-transplant with medical or surgical therapy are warranted.

Adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA)

Recommendations for referral and listing:
- ❖ Diffuse parenchymal tumor involvement causing lung restriction and significant respiratory compromise;
- ❖ Significantly reduced quality of life;
- ❖ Failure of conventional medical therapies.

With regard to transplantation, the following evaluation and management is suggested:

- ❖ Prior to listing for lung transplantation, the tumor should be biopsied and/or tissue from a previous resection thoroughly examined to exclude more invasive disease;
- ❖ Patients should undergo thorough staging with chest and abdominal computer tomography, brain magnetic resonance imaging, bone scanning and positron emission tomography. These tests should be repeated regularly (every 3 months is suggested) to detect metastases that would result in delisting of the patient;
- ❖ At the time of lung transplantation, a backup recipient should be available so that if mediastinal nodal involvement or spread beyond the pleura is detected, the operation should be discontinued and a substitute recipient should receive the lungs.

The rationale of lung transplantation for AIS and MIA [either pure lepidic growth (AIS) or predominant lepidic growth] was developed when these tumors were referred to as diffuse bronchioloalveolar carcinoma (BAC). Regardless of nomenclature, lung transplant has been performed based on the tumor being confined to the lungs. While survival after resection for localized disease is quite good, the results of chemotherapy for diffuse, bilateral disease is poor with survival beyond two years from the time of diagnosis quite uncommon. Hence, some centers have performed lung transplantation in patients with diffuse BAC (90,91).

In 2004, a report by de Perrot and colleagues (90) characterized the international experience with lung transplantation for BAC. The survival of patients undergoing lung transplantation and heart/lung transplantation for BAC at 5 and 10 years (26 patients) was 39% and 31% respectively, as compared to the survival reported by the International Society for Heart and Lung Transplantation in the 2013 Registry report (53) where the survival at 5 years was 53% and at 10 years was 31%.

One of the major concerns about lung transplantation for BAC is the incidence of recurrent tumor. In the survey by de Perrot (90), of the 22 patients that survived the operation, 13 (59%) developed a recurrence of BAC between 5 and 49 months after transplantation. Zorn and colleagues (91) also saw a high recurrence rate in the small series, where tumor recurred in 6 of 8 patients. One of the interesting features of the recurrences is the demonstration of their recipient origin (92,93), suggesting that the mechanism of the recurrence may be contamination of the donor lungs from retention of malignant cells in the airways after excision of the recipient lungs.

The survival of patients after lung transplantation for BAC based on the small worldwide experience appears to be marginally inferior to that of lung transplantation for other conditions in the current era. Nevertheless, compared to the natural history of diffuse and bilateral BAC and the ineffectiveness of chemotherapy, outcomes after lung transplantation are far superior to the natural history of the disease, despite high recurrence rates of BAC after lung transplantation.

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Footnote

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References

1. Orens JB, Estenne M, Arcasoy S, et al. International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2006;25:745-55.

2. International guidelines for the selection of lung transplant candidates. The American Society for Transplant Physicians (ASTP)/American Thoracic Society(ATS)/ European Respiratory Society(ERS)/International Society for Heart and Lung Transplantation(ISHLT). Am J Respir Crit Care Med 1998;158:335-9.

3. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.

4. Shigemura N, Bhama J, Gries CJ, et al. Lung transplantation in patients with prior cardiothoracic surgical procedures. Am J Transplant 2012;12:1249-55.

5. Reynaud-Gaubert M, Mornex JF, Mal H, et al. Lung transplantation for lymphangioleiomyomatosis: the French experience. Transplantation 2008;86:515-20.

6. Detterbeck FC, Egan TM, Mill MR. Lung transplantation after previous thoracic surgical procedures. Ann Thorac Surg 1995;60:139-43.

7. Dusmet M, Winton TL, Kesten S, et al. Previous intrapleural procedures do not adversely affect lung transplantation. J Heart Lung Transplant 1996;15:249-54.

8. Curtis HJ, Bourke SJ, Dark JH, et al. Lung transplantation outcome in cystic fibrosis patients with previous pneumothorax. J Heart Lung Transplant 2005;24:865-9.

9. Senbaklavaci O, Wisser W, Ozpeker C, et al. Successful lung volume reduction surgery brings patients into better condition for later lung transplantation. Eur J Cardiothorac Surg 2002;22:363-7.

10. Shigemura N, Gilbert S, Bhama JK, et al. Lung transplantation after lung volume reduction surgery. Transplantation 2013;96:421-5.

11. Cypel M, Keshavjee S. Extracorporeal life support as a bridge to lung transplantation. Clin Chest Med 2011;32:245-51.

12. de Perrot M, Granton JT, McRae K, et al. Outcome of patients with pulmonary arterial hypertension referred for lung transplantation: a 14-year single- center experience. J Thorac Cardiovasc Surg 2012;143:910-8.

13. Strueber M. Bridges to lung transplantation. Curr Opin Organ Transplant 2011;16:458-61.

14. Gottlieb J, Warnecke G, Hadem J, et al. Outcome of critically ill lung transplant candidates on invasive respiratory support. Intensive Care Med 2012;38:968-75.

15. de Perrot M, Granton JT, McRae K, et al. Impact of extracorporeal life support on outcome in patients with idiopathic pulmonary arterial hypertension awaiting lung transplantation. J Heart Lung Transplant 2011;30:997-1002.

16. Vermeijden JW, Zijlstra JG, Erasmus ME, et al. Lung transplantation for ventilator-dependent respiratory failure. J Heart Lung Transplant 2009;28:347-51.

17. Diaz-Guzman E, Hoopes CW, Zwischenberger JB. The evolution of extracorporeal life support as a bridge to lung transplantation. ASAIO J 2013;59:3-10.

18. Javidfar J, Brodie D, Iribarne A, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation and recovery. J Thorac Cardiovasc Surg 2012;144:716-21.

19. Toyoda Y, Bhama JK, Shigemura N, et al. Efficacy of extracorporeal membrane oxygenation as a bridge to lung transplantation. J Thorac Cardiovasc Surg 2013;145:1065-70; discussion 1670-1.

20. Shafii AE, Mason DP, Brown CR, et al. Growing experience with extracorporeal membrane oxygenation as a bridge to lung transplantation. ASAIO J 2012;58:526-9.

21. Hoopes CW, Kukreja J, Golden J, et al. Extracorporeal membrane oxygenation as a bridge to pulmonary transplantation. J Thorac Cardiovasc Surg 2013;145:862-

7; discussion 867-8.

22. Bittner HB, Lehmann S, Rastan A, et al. Outcome of extracorporeal membrane oxygenation as a bridge to lung transplantation and graft recovery. Ann Thorac Surg 2012;94:942-9; author reply 949-50.

23. Fuehner T, Kuehn C, Hadem J, et al. Extracorporeal membrane oxygenation in awake patients as bridge to lung transplantation. Am J Respir Crit Care Med 2012;185:763-8.

24. Snyder JJ, Salkowski N, Skeans M, et al. The equitable allocation of deceased donor lungs for transplant in children in the United States. Am J Transplant 2014;14:178-83.

25. King TE, Jr., Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med 2014;370:2083-92.

26. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788-824.

27. Brown AW, Shlobin OA, Weir N, et al. Dynamic patient counseling: a novel concept in idiopathic pulmonary fibrosis. Chest 2012;142:1005-10.

28. du Bois RM, Weycker D, Albera C, et al. Six-minute-walk test in idiopathic pulmonary fibrosis: test validation and minimal clinically important difference. Am J Respir Crit Care Med 2011;183:1231-7.

29. Collard HR, King TE Jr, Bartelson BB, et al. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2003;168:538-42.

30. du Bois RM, Weycker D, Albera C, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011;184:459-66.

31. Flaherty KR, Andrei AC, Murray S, et al. Idiopathic pulmonary fibrosis: prognostic value of changes in physiology and six-minute-walk test. Am J Respir Crit Care Med 2006;174:803-9.

32. Lederer DJ, Arcasoy SM, Wilt JS, et al. Six-minute-walk distance predicts waiting list survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2006;174:659-64.

33. King TE Jr, Tooze JA, Schwarz MI, et al. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med 2001;164:1171-81.

34. Bongartz T, Nannini C, Medina-Velasquez YF, et al. Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. Arthritis and rheumatism 2010;62:1583-91.

35. Bouros D, Wells AU, Nicholson AG, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. Am J Respir Crit Care Med 2002;165:1581-6.

36. Rosenbluth DB, Wilson K, Ferkol T, et al. Lung function decline in cystic fibrosis patients and timing for lung transplantation referral. Chest 2004;126:412-9.

37. Kerem E, Reisman J, Corey M, et al. Prediction of mortality in patients with cystic fibrosis. N Engl J Med 1992;326:1187-91.

38. Mayer-Hamblett N, Rosenfeld M, Emerson J, et al. Developing cystic fibrosis lung transplant referral criteria using predictors of 2-year mortality. Am J Respir Crit Care Med 2002;166:1550-5.

39. Augarten A, Akons H, Aviram M, et al. Prediction of mortality and timing of referral for lung transplantation in cystic fibrosis patients. Pediatric transplantation 2001;5:339-42.

40. Milla CE, Warwick WJ. Risk of death in cystic fibrosis patients with severely compromised lung function. Chest 1998;113:1230-4.

41. Liou TG, Adler FR, Cahill BC, et al. Survival effect of lung transplantation among patients with cystic fibrosis. JAMA 2001;286:2683-9.

42. Kadikar A, Maurer J, Kesten S. The six-minute walk test: a guide to assessment for lung transplantation. J Heart Lung Transplant 1997;16:313-9.

43. Tuppin MP, Paratz JD, Chang AT, et al. Predictive utility of the 6-minute walk distance on survival in patients awaiting lung transplantation. J Heart Lung Transplant 2008;27:729-34.

44. Venuta F, Rendina EA, Rocca GD, et al. Pulmonary hemodynamics contribute to indicate priority for lung transplantation in patients with cystic fibrosis. J Thorac Cardiovasc Surg 2000;119:682-9.

45. Venuta F, Tonelli AR, Anile M, et al. Pulmonary hypertension is associated with higher mortality in cystic fibrosis patients awaiting lung transplantation. J Cardiovasc Surg (Torino) 2012;53:817-20.

46. Esther CR Jr, Esserman DA, Gilligan P, et al. Chronic Mycobacterium abscessus infection and lung function decline in cystic fibrosis. J Cyst Fibros 2010;9:117-23.

47. Flume PA, Mogayzel PJ Jr, Robinson KA, et al. Cystic fibrosis pulmonary guidelines: pulmonary complications: hemoptysis and pneumothorax. Am J Respir Crit Care

Med 2010;182:298-306.

48. Lobo LJ, Chang LC, Esther CR Jr, et al. Lung transplant outcomes in cystic fibrosis patients with pre-operative Mycobacterium abscessus respiratory infections. Clin Transplant 2013;27:523-9.

49. Chernenko SM, Humar A, Hutcheon M, et al. Mycobacterium abscessus infections in lung transplant recipients: the international experience. J Heart Lung Transplant 2006;25:1447-55.

50. De Soyza A, Corris PA. Lung transplantation and the Burkholderia cepacia complex. J Heart Lung Transplant 2003;22:954-8.

51. De Soyza A, McDowell A, Archer L, et al. Burkholderia cepacia complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. Lancet 2001;358:1780-1.

52. De Soyza A, Meachery G, Hester KL, et al. Lung transplantation for patients with cystic fibrosis and Burkholderia cepacia complex infection: a single-center experience. J Heart Lung Transplant 2010;29:1395-404.

53. Yusen RD, Christie JD, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: thirtieth adult lung and heart-lung transplant report--2013; focus theme: age. J Heart Lung Transplant 2013;32:965-78.

54. Martinez FJ, Foster G, Curtis JL, et al. Predictors of mortality in patients with emphysema and severe airflow obstruction. Am J Respir Crit Care Med 2006;173:1326-34.

55. Celli BR, Cote CG, Marin JM, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med 2004;350:1005-12.

56. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 1995;152:S77-121.

57. Soler-Cataluña JJ, Martinez-Garcia MA, Roman Sanchez P, et al. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. Thorax 2005;60:925-31.

58. Soler-Cataluña JJ, Martinez-Garcia MA, Sanchez LS, et al. Severe exacerbations and BODE index: two independent risk factors for death in male COPD patients. Respir Med 2009;103:692-9.

59. Connors AF Jr, Dawson NV, Thomas C, et al. Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments). Am J Respir Crit Care Med 1996;154:959-67.

60. Lahzami S, Bridevaux PO, Soccal PM, et al. Survival impact of lung transplantation for COPD. Eur Respir J 2010;36:74-80.

61. Marchand E. The BODE index as a tool to predict survival in COPD lung transplant candidates. Eur Respir J 2010;36:1494-5; author reply 1495.

62. Kreider M, Hadjiliadis D, Kotloff RM. Candidate selection, timing of listing, and choice of procedure for lung transplantation. Clin Chest Med 2011;32:199-211.

63. Gottlieb J. Lung transplantation for interstitial lung diseases and pulmonary hypertension. Semin Respir Crit Care Med 2013;34:281-7.

64. Conte JV, Gaine SP, Orens JB, et al. The influence of continuous intravenous prostacyclin therapy for primary pulmonary hypertension on the timing and outcome of transplantation. J Heart Lung Transplant 1998;17:679-85.

65. Robbins IM, Christman BW, Newman JH, et al. A survey of diagnostic practices and the use of epoprostenol in patients with primary pulmonary hypertension. Chest 1998;114:1269-75.

66. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation 2010;122:164-72.

67. Eberlein M, Garrity ER, Orens JB. Lung allocation in the United States. Clin Chest Med 2011;32:213-22.

68. Egan TM, Murray S, Bustami RT, et al. Development of the new lung allocation system in the United States. Am J Transplant 2006;6:1212-27.

69. Kawut SM, Lederer DJ, Keshavjee S, et al. Outcomes after lung retransplantation in the modern era. Am J Respir Crit Care Med 2008;177:114-20.

70. Novick RJ, Stitt LW, Al-Kattan K, et al. Pulmonary retransplantation: predictors of graft function and survival in 230 patients. Pulmonary Retransplant Registry. Ann Thorac Surg 1998;65:227-34.

71. Kawut SM. Lung retransplantation. Clin Chest Med 2011;32:367-77.

72. Strueber M, Fischer S, Gottlieb J, et al. Long-term outcome after pulmonary retransplantation. J Thorac Cardiovasc Surg 2006;132:407-12.

73. Aigner C, Jaksch P, Taghavi S, et al. Pulmonary retransplantation: is it worth the effort? A long-term analysis of 46 cases. J Heart Lung Transplant 2008;27:60-5.

74. Choong CK, Sweet SC, Guthrie TJ, et al. Repair

of congenital heart lesions combined with lung transplantation for the treatment of severe pulmonary hypertension: a 13-year experience. J Thorac Cardiovasc Surg 2005;129:661-9.

75. Faro A, Mallory GB, Visner GA, et al. American Society of Transplantation executive summary on pediatric lung transplantation. Am J Transplant 2007;7:285-92.

76. Fadel E, Mercier O, Mussot S, et al. Long-term outcome of double-lung and heart-lung transplantation for pulmonary hypertension: a comparative retrospective study of 219 patients. Eur J Cardiothorac Surg 2010;38:277-84.

77. Januszewska K, Malec E, Juchem G, et al. Heart-lung transplantation in patients with pulmonary atresia and ventricular septal defect. J Thorac Cardiovasc Surg 2009;138:738-43.

78. Wolf JH, Sulewski ME, Cassuto JR, et al. Simultaneous thoracic and abdominal transplantation: can we justify two organs for one recipient? Am J Transplant 2013;13:1806-16.

79. Couetil JP, Houssin DP, Soubrane O, et al. Combined lung and liver transplantation in patients with cystic fibrosis. A 4 1/2-year experience. J Thorac Cardiovasc Surg 1995;110:1415-22; discussion 1422-3.

80. Grannas G, Neipp M, Hoeper MM, et al. Indications for and outcomes after combined lung and liver transplantation: a single-center experience on 13 consecutive cases. Transplantation 2008;85:524-31.

81. Cassuto JR, Reese PP, Sonnad S, et al. Wait list death and survival benefit of kidney transplantation among nonrenal transplant recipients. Am J Transplant 2010;10:2502-11.

82. Lonze BE, Warren DS, Stewart ZA, et al. Kidney transplantation in previous heart or lung recipients. Am J Transplant 2009;9:578-85.

83. Arnon R, Annunziato RA, Miloh T, et al. Liver and combined lung and liver transplantation for cystic fibrosis:

analysis of the UNOS database. Pediatr Transplant 2011;15:254-64.

84. Barshes NR, DiBardino DJ, McKenzie ED, et al. Combined lung and liver transplantation: the United States experience. Transplantation 2005;80:1161-7.

85. Praseedom RK, McNeil KD, Watson CJ, et al. Combined transplantation of the heart, lung, and liver. Lancet 2001;358:812-3.

86. Yi SG, Burroughs SG, Loebe M, et al. Combined lung and liver transplantation: Analysis of a single-center experience. Liver Transpl 2014;20:46-53.

87. Pirenne J, Verleden G, Nevens F, et al. Combined liver and (heart-)lung transplantation in liver transplant candidates with refractory portopulmonary hypertension. Transplantation 2002;73:140-2.

88. Saggar R, Khanna D, Furst DE, et al. Systemic sclerosis and bilateral lung transplantation: a single centre experience. Eur Respir J 2010;36:893-900.

89. Sottile PD, Iturbe D, Katsumoto TR, et al. Outcomes in systemic sclerosis- related lung disease after lung transplantation. Transplantation 2013;95:975-80.

90. de Perrot M, Chernenko S, Waddell TK, et al. Role of lung transplantation in the treatment of bronchogenic carcinomas for patients with end-stage pulmonary disease. J Clin Oncol 2004;22:4351-6.

91. Zorn GL Jr, McGiffin DC, Young KR Jr, et al. Pulmonary transplantation for advanced bronchioloalveolar carcinoma. J Thorac Cardiovasc Surg 2003;125:45-8.

92. Garver RI Jr, Zorn GL, Wu X, et al. Recurrence of bronchioloalveolar carcinoma in transplanted lungs. N Engl J Med 1999;340:1071-4.

93. Gómez-Román JJ, Del Valle CE, Zarrabeitia MT, et al. Recurrence of bronchioloalveolar carcinoma in donor lung after lung transplantation: microsatellite analysis demonstrates a recipient origin. Pathol Int 2005;55:580-4.

Recipient selection process and listing for lung transplantation

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Abstract: Lung transplantation remains the ultimate treatment option for selected patients with end-stage (cardio) pulmonary disease. Given the current organ shortage, it is without any doubt that careful selection of potential transplant candidates is essential as this may greatly influence survival after the procedure. In this paper, we will review the current guidelines for referral and listing of lung transplant candidates in general, and in more depth for the specific underlying diseases. Needless to state that these are not absolute guidelines, and that decisions depend upon center's activity, waiting list, etc. Therefore, every patient should be discussed with the transplant center before any definite decision is made to accept or decline a patient for lung transplantation.

Keywords: Lung transplantation; referral guidelines; candidate selection

General indications for lung transplantation

Lung transplantation is performed with increasing numbers all over the world and in the most recent official lung and heart-lung transplant registry report released by the International Society for Heart and lung Transplantation (1), it was mentioned that in 2014 some 4,000 lung transplantation have been performed worldwide, of which 75% were double lung transplants. In this report, which accumulated over 50,000 lung transplantations from 1995 till June 2015, COPD with and without alpha1 antitrypsin deficiency accounted for 36.5%, interstitial lung disease [including idiopathic pulmonary fibrosis (IPF)] for 29.7%, cystic fibrosis (CF) for 15.8%, non-CF bronchiectasis for 2.7%, pulmonary hypertension for 4.4%, retransplantation for 4.1%, and some less common indications such as sarcoidosis, lymphangioleiomyomatosis (LAM), obliterative bronchiolitis, etc. for 6.8% of the total number. These data are gathered from 134 collaborating centers all over the world. In our own center in Leuven (Belgium), the underlying diseases for lung transplantation are shown in *Figure 1*, and are quite representative for the registry data.

From the beginning of our centers activity, we have used the available guidelines for the selection of lung transplant candidates, although the first international guidelines were only published in 1998 by Maurer *et al.* This was a joint guidelines paper, produced by ATS, ISHLT, AST and ERS and simultaneously published in *the Journal of Heart and Lung Transplantation*, *Heart Lung* and *Transplantation* (2-4). This paper focused on general medical conditions which impact on eligibility for lung transplantation and clearly indicated an age limit of up to 55 years for heart-lung, 65 years for single lung and 60 years for double lung transplantation. Also disease specific criteria were already mentioned.

In the next guidelines paper, published in *the Journal of Heart and Lung Transplantation* in 2006, by Orens *et al.* (5), it was clearly stated that evolving technology and advances in medical knowledge mandated a need for an update. In this revision, age >65 years was only considered as a relative contra indication, given the enhanced experience with such patients. The paper also made a distinction between referral guidelines and transplantation guidelines, which was quite elegant to use at the time. There were no new criteria for
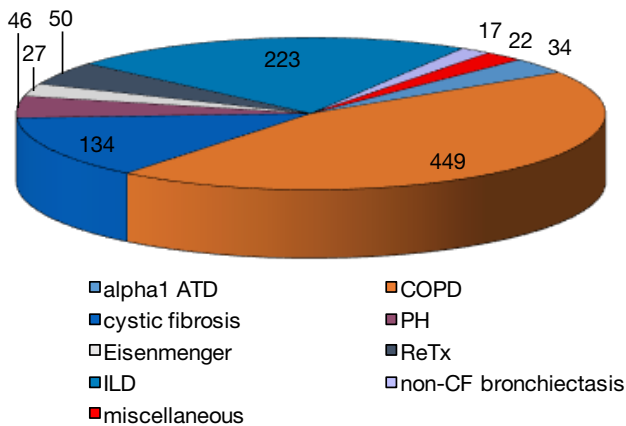
Figure 1 Indications for lung and heart-lung transplantation at the University Hospital Leuven (n=1,002 procedures) from July 1991 till June 2017.

pediatric transplantation nor for retransplantation.

The most recent update of the guidelines was published in 2014, by Weil *et al.* and will form the further basis for this chapter (6).

As the mortality rate after lung transplantation relative to other solid-organ transplants is high and the availability of donor lungs remains limited, lung transplantation should be offered to those in whom a survival benefit can be expected. Overall median survival in most recent reports is 5.8 years with an unadjusted survival rate at 5 years of 54% (1). However, the median survival rate according to the underlying pulmonary disease is very different, varying from 2.8 years after retransplantation to 8.9 years for CF.

Thus, selected adult patients should have chronic, end-stage lung diseases and meet the following criteria:

(I)   High risk of death (>50%) within 2 years if lung transplantation is not performed;

(II)   High likelihood (>80%) of surviving at least 90 days after lung transplantation;

(III)   High likelihood (>80%) of 5-year post-transplant survival;

(IV)   No other treatment option possible/available.

Contraindications

The ISHLT's 2014 guidelines include absolute and relative contraindications. These are of course to be interpreted with some caution, as experienced centers may have other contra indications compared to starting centers. What is really to be considered is the fact that in the below mentioned conditions, there should at least be in depth discussion with the transplant team and the patient, whether a lung transplantation is indeed the right option for this particular patient. Some of these absolute contraindications may also be temporary as for instance a patient may lose weight and decrease a BMI to <35 kg/m^2, or an active *Mycobacterium tuberculosis* infection may be treated for several months before reconsidering the patient for lung transplantation.

Absolute contraindications to lung transplantation

(I)   Recent history of malignancy. A 2-year disease-free interval and a low predicted risk of recurrence may be acceptable, for instance, in localized squamous or basal cell skin cancer, appropriately treated. However, a 5-year disease-free interval is required in most cases, particularly for patients with a history of hematologic malignancy, sarcoma, melanoma, or cancers of the breast, bladder, or kidney. For patients with a history of bronchial carcinoma, for instance, the risk of recurrence may remain too high. A specific condition may be localized prostate cancer, even diagnosed at the time of pre transplant work up, with a Gleason score of max. 3+3 may be acceptable in some patients, although data remain scarce.

(II)   Untreatable significant dysfunction of another major organ system (e.g., heart, liver, kidney, or brain) unless combined organ transplantation can be performed. Several combined organ transplantations have been performed worldwide, with variable outcome, again depending on the experience of the center. Typical examples are combined liver-lung, lung-kidney, heart-lung and liver, lung, kidney, pancreas. Survival with lung-liver in CF patients is reported to be comparable to transplantation of lungs only (7,8).

(III)   Uncorrected atherosclerotic disease with suspected or confirmed end-organ ischemia or dysfunction and/or coronary artery disease not amenable to revascularization.

(IV)   Acute medical instability, including, but not limited to, acute sepsis, myocardial infarction, and liver failure. In our own center one patient was transplanted with end-stage COPD and drug-induced acute liver failure. She is doing well >3 years after the procedure (9). This illustrates that such combined transplantations may be feasible, but

should be very well discussed before to proceed.

(V) Uncorrectable bleeding diathesis.

(VI) Chronic infection with highly virulent and/or resistant microbes that are poorly controlled pre-transplant. Human immunodeficiency virus (HIV) and hepatitis B or C are no longer considered as absolute contra-indications, provided patients are treated for HIV and there is no viremia for hepatitis B and C (10,11).

(VII) Evidence of active *Mycobacterium tuberculosis* infection.

(VIII) Significant chest wall or spinal deformity expected to cause severe restriction after transplantation. Morbus Bechterew may be one of these specific conditions. If indeed the mobility of the thoracic cage is severely restricted pretransplant, many problems may arise after transplantation, such as difficult weaning and restrictive pulmonary function with ongoing dysfunctionality.

(IX) Class II or III obesity [body mass index (BMI) ≥35.0 kg/m^2].

(X) Current non-adherence to medical therapy or a history of repeated or prolonged episodes of non-adherence to medical therapy that are perceived to increase the risk of non-adherence after transplantation. This is sometimes difficult to assess, but is very important as it was recently shown that adherence after transplantation may also be problematic, although this was less problematic after lung transplantation (12).

(XI) Psychiatric or psychologic conditions associated with the inability to cooperate with the medical/allied health care team and/or adhere with complex medical therapy.

(XII) Absence of an adequate or reliable social support system.

(XIII) Severely limited functional status with poor rehabilitation potential.

(XIV) Substance abuse or dependence (e.g., alcohol, tobacco, marijuana, or other illicit substances). In many cases, convincing evidence of risk reduction behaviors, such as meaningful and/or long-term participation in therapy for substance abuse and/or dependence, should be required before offering lung transplantation. Serial blood and urine testing can be used to verify abstinence from substances that are of concern. Prior tobacco smoking is common in patients who have end-stage lung disease, especially COPD and IPF. It is generally assumed that an abstinence period of 6 months might be sufficient before transplantation (or listing), although we recently demonstrated that the time of smoking cessation before transplantation inversely correlated with resumption of smoking after lung transplantation. Moreover, other smoking family members in the household of the patient are another risk factor to resume smoking afterwards. Therefore, presently we also try to convince everyone living in the same house to quit smoking (13).

Relative contraindications

Relative contraindications remain a matter of debate, indeed what is relative in one center may be absolute in another center. This greatly depends on the experience of the center. All these relative contraindications should therefore be interpreted with caution, and too many relative contraindications may become an absolute contra indication for lung transplantation.

(I) Age >65 years in association with low physiologic reserve and/or other relative contraindications. It is evident from the literature that older patients have a worse outcome. This was clearly identified in the ISHLT registry report from 2013 (14) which focused on age. Between 2003 and 2012, 19,930 lung transplants were registered, of which 10% were in patients >65 years of age. Survival not only varies by era, and by underlying disease, within each diagnostic group, older patients had a worse survival and in general the 5-year survival in >65 years was 38%, compared to 46% for those between 60–65 years and 52% to 57% for those <60 years (14). Of course, age is not the only factor that counts, also frailty of the patient is important. A frail patient <60 years may have a worse prognosis compared to a non-frail >65 years old. All factors should indeed be taken into account when considering an older patient for lung transplantation. Whether older patients may benefit more from a single than from a double lung remains a matter of debate, as there are a lot of contradictory studies.

(II) Class I obesity (BMI: 30.0–34.9 kg/m^2), particularly truncal (central) obesity and progressive or severe malnutrition, with a BMI <14. Although there is evidence from large databases that as well overweight as underweight patients suffer from

a worse survival compared to normal weight patients (15,16), the debate is still going on. We have recently investigated the role of BMI on outcome after lung transplantation in 546 LT recipients, of which 28% had BMI <18.5 kg/m^2. Underweight resulted in similar survival (P=0.28) compared to the normal weight group. Significantly higher mortality was found in overweight (P=0.016) and obese patients (P=0.031) compared with the normal-weight group. Subanalysis of either underweight (P=0.19) or obese COPD patients (P=0.50) did not reveal worse survival. In patients with interstitial lung disease, obesity was associated with increased mortality (P=0.031) compared to the normal-weight group. In CF patients, underweight was not associated with a higher mortality rate (P=0.12) compared to the normal-weight group (17).

(III)   Severe, symptomatic osteoporosis. This might indeed result in further vertebral fractures that may compromise breathing, coughing and rehabilitation after lung transplantation, leading to a more complicated postoperative course.

(IV)   Extensive prior chest surgery with lung resection. This greatly depends on the experience of the surgeons and the center. In a rather old series investigating this subject, it was found that in carefully selected cases, previous thoracic surgery had no major impact on lung transplantation outcome. The perioperative risk and the transfusion requirements were not elevated compared to patients without previous thoracic surgery. However, the surgical procedure itself was more difficult (18).

(V)   Mechanical ventilation and/or extracorporeal life support (ECLS). Nowadays, there is increasing experience with extra corporeal membrane oxygenation (ECMO) as a bridge to lung transplantation; In experienced centers, this has little impact on survival, however, patients need to be carefully evaluated and the center volume seems very important and does impact on survival (19). Recently, there is emerging evidence that awakes ECMO which enables further rehabilitation, may lead to improved results after lung transplantation, with a 2-year survival of 81% (20).

(VI)   Colonization or infection with highly resistant or highly virulent bacteria, fungi, and certain strains of mycobacteria (e.g., chronic extrapulmonary infection expected to worsen after transplantation). This again is highly dependent on the experience of the center. A classic example is the presence of *Mycobacterium abscessus* in CF patients, which is in some centers an absolute contraindication, whereas in others it is a relative one (21,22). Patients who are infected with *Burkholderia cenocepacia* or *Burkholderia* gladioli are a particular challenge for lung transplantation and could be considered for transplantation if the infection is sufficiently treated preoperatively and if there is a reasonable expectation for adequate control postoperatively (23,24). For patients infected with hepatitis B and/or C, a lung transplant can be considered when there are no significant clinical, radiologic, or biochemical signs of cirrhosis or portal hypertension; moreover, the patients should be stable on appropriate therapy. For patients infected with HIV, a lung transplant can be considered in those with controlled disease with undetectable HIV-RNA, and compliant on combined anti-retroviral therapy (25). In general, patients with these infections should be evaluated by a transplant center with significant experience managing these infections, and patients should be informed of the increased risk of transplantation.

(VII)   Atherosclerotic disease burden without end-organ disease. With regard to coronary artery disease, some patients will be candidates for percutaneous coronary intervention or simultaneous coronary artery bypass graft (CABG). The preoperative evaluation, type of coronary stent used (bare metal *vs.* drug eluting), and degree of coronary artery disease that is accepted vary among transplant centers. In our own experience, we treated 23 patients out of a total of 775 isolated lung transplantation procedures with either one or two stents (n=20) or simultaneous CABG (n=3). The survival rates were similar in both groups, illustrating that preoperative or intraoperative correction of the coronary stenosis results in a similar outcome as in patients with no coronary stenosis.

(VIII)   Other medical conditions that have not resulted in end-stage organ damage, such as diabetes mellitus, systemic arterial hypertension, epilepsy, central venous obstruction, peptic ulcer disease, or gastroesophageal reflux, should be optimally

Table 1 Calculation of the BODE index (37)

Variable	Points on BODE index			
	0	1	2	3
FEV_1, % pred.	>65	50–65	35–49	<35
Dyspnea, MRC	0–1	2	3	4
6 MWD (meters)	>350	250–349	150–249	<149
BMI (kg/m^2)	<21	>21	–	–

FEV_1, forced expiratory volume in the first second; MRC, medical research council; MWD, minutes walk distance; BMI, body mass index.

treated before transplantation. Gastroesophageal reflux is highly prevalent in lung transplantation candidates and is often asymptomatic, requiring invasive testing for diagnosis (26); moreover, in general, GERD worsens after transplantation (27). GERD is accepted to be a risk factor for allograft dysfunction after lung transplantation, especially acute rejection (28) and bronchiolitis obliterans syndrome (BOS) (29,30). Treatment may require surgical intervention, such as a Nissen fundoplication. Whether a possible treatment should be performed before or after transplantation, especially in the prevention of BOS, remains a matter of debate. Indeed preventive surgery may lead to a better postoperative FEV_1, but does not necessarily impact on the occurrence of BOS (31-34). A special consideration is the patients with scleroderma and esophageal dysfunction/motility with reflux. Although this condition was initially regarded as a contra indication for lung transplantation, at least in some centers, recent evidence suggests that in selected patients, even those with gross reflux and esophageal dysfunction, results are acceptable, with a 5-year survival of 70%. Also, the prevalence of BOS was comparable to non-scleroderma patients (35).

Specific disease related referral and transplantation criteria

Because of the existence of a transplantation window, this means the time between activation on the waiting list and the transplantation procedure, which varies according to the underlying condition and per transplant center, it is obvious that patients need to be referred in time. As a consequence,

a differentiation has been made between referral criteria for lung transplantation (meaning at the start of the transplant window) and transplantation criteria. This difference between referral and transplantation criteria was first used in the 2006 guidelines (6), and again in the revised 2014 guidelines (7). This is of utmost importance, since, in general, the waiting time varies according to the underlying disease which is an important factor to calculate the lung allocation score (LAS). Indeed the LAS, which means the urgency of a transplantation procedure, is much higher for a patient with for instance IPF compared to a stable COPD patient, illustrating that a patient with IPF will have a shorter waiting time. This all needs to be taken into account when referring a patient to a local transplant center (36).

Chronic obstructive pulmonary disease and alpha1-antitrypsin deficiency

COPD remains the most prevalent indication for lung transplantation, with 36.5% of all procedures being performed worldwide between Jan 1995 and Jun 2015. Of these, 57.3% are double lung transplantations (1). Although COPD should constitute a simple diagnosis, there is much heterogeneity which makes it often difficult to adhere to strict transplantation criteria. This is very well illustrated by the recent 2017 GOLD guidelines, which not only stage COPD based on FEV_1 but also on dyspnea, number of (severe) exacerbations and co morbidities.

In general, prognosis of COPD depends on the severity of the airway obstruction, breathlessness, number of exacerbations and functional limitation. This is reflected in the BODE index, which points to survival rates (*Table 1*) (37). Although the BODE index may be used as prognosticator for COPD patients in general, there is debate whether this can also be used in selected COPD patients who may qualify for lung transplantation (38). Indeed, in this latter population, there is in general less comorbidity, which may impact on survival. Nevertheless, BODE index has been used in COPD patients who qualify for lung transplantation and seems to be a help to identify suitable transplant candidates with COPD (39,40). A BODE score of 7–10 was associated with a mortality of 80% at 4 years, whereas a score of 5–6 conferred a mortality of 60% at 4 years, and proved to be a better indicator of survival than the spirometric staging system. Indeed, Lahzami *et al.* evaluated the role of the BODE score in lung transplantation for COPD and demonstrated that most patients with COPD had an individual survival benefit from lung transplantation regardless of their pre-transplant

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Table 2 Referral and transplantation guidelines for COPD (6)

Referral guidelines
Disease progression, despite maximal treatment including medication, pulmonary rehabilitation, and oxygen therapy
Patient is not a candidate for endoscopic or surgical LVRS. Simultaneous referral of patients with COPD for both lung transplant and LVRS evaluation is appropriate
BODE index of 5 to 6
$PaCO_2$ >45 mmHg or >6.6 kPa and/or PaO_2 <60 mmHg or <8 kPa
FEV_1 <25% predicted
Transplantation guidelines
BODE index ≥7
FEV_1 <15% to 20% predicted
Three or more severe exacerbations during the preceding year
One severe exacerbation with acute hypercapnic respiratory failure
Moderate to severe pulmonary hypertension

BODE score, although a global survival benefit was only seen in patients with a BODE score ≥7, suggesting that this is the appropriate population to transplant (41).

Exacerbation frequency and the severity of exacerbations is also a known prognosticator in COPD; indeed, the presence of ≥3 exacerbations/year negatively affects survival in patients with COPD (42). The increased mortality risk is independent of the BODE index (43). Acute hypercapnic respiratory failure increases the in-hospital mortality to 41%, and to a further 43% and 49% after 1 and 2 years in survivors (44). In another cohort, the 1-year mortality in patients who needed non-invasive ventilation during an acute COPD exacerbation was 30% (45). Specific referral and transplantation guidelines for patients with COPD are summarized in *Table 2*.

As already stated in the general indications for lung transplantation, there should no other treatment option be available, besides lung transplantation. This off course includes rehabilitation and also the possibility of lung volume reduction (LVR), either endoscopically (E) or surgically (S). According to the NETT trial, patients with an FEV_1 of less than 20%, as well as a diffusing capacity of the lungs for carbon monoxide (D_{LCO}) of less than 20% or homogenous emphysema, are at high risk for death with LVRS and are not eligible for this surgical procedure (46). On the other hand, only a very selected subgroup of patients with upper lobe predominant emphysema, poor exercise capacity and no major contraindication for lung surgery may be suitable candidates for LVRS. With these restrictions, LVRS is still rarely performed and research has focused on bronchoscopic LVR procedures.

One-way endobronchial valve (EBV) placement is the best studied approach and is targeted to the most emphysematous destroyed lung lobe. The first trials showed some benefits on pulmonary function parameters but the results were considered as not clinically meaningful. However, post-hoc analyses demonstrated that patients with an intact interlobular fissure on HRCT scan experienced the best outcome following EBV implantation (47). The STELVIO study compared EBV treatment (n=34) versus standard care (n=34) in a selected group of patients with severe emphysema and absence of collateral ventilation. In the intention-to-treat population, a statistical and clinical significant improvement of FEV_1 (Δ140 mL or Δ17.8%), forced vital capacity (FVC) (Δ347 mL or Δ14.4%) and 6-minute walking distance (6 MWD) (Δ74 m or Δ23.3%) was observed after 6 months. When focusing on the per-protocol analysis, [intervention (n=25) *vs.* control (n=33)], the effect was even larger [+191 mL for FEV_1 (95% CI, 109–272 mL), + 442 mL for FVC (95% CI, 215–668 mL)] and +106 m on 6 MWT (95% CI, 80–133 m) and also resulted in major changes in quality of life (–14.9 difference on SGRQ). A similar magnitude of effects was observed in the standard treatment group that switched to the intervention after 6 months (46). Moreover, data on patients in further follow-up (n=40/64) confirmed sustained benefits with 65%, 63% and 75% responders after 1 year taking into account the minimal clinical important differences (MCID) for respectively FEV_1, SGRQ and 6 MWT (48).

In that respect, LVRS or LVRE might deviate some selected COPD patients from lung transplantation, or on the other hand may temporarily improve these patients which may result in better rehabilitation potential and in a better outcome after a subsequent lung transplantation.

Diffuse parenchymal lung disease (DPLD)

DPLD constitutes about 30–35% of all indications for lung transplantation, with IPF being the largest indication amongst all DPLD (1). The other DPLD for which transplants are also performed include LAM, histiocytosis X, sarcoidosis, and collagen vascular disease-associated interstitial lung diseases, such as scleroderma, polymyositis

Table 3 Referral and transplantation guidelines in IPF and other DPLD (6)

Referral guidelines
Histopathologic or radiographic evidence of UIP or fibrosing NSIP, regardless of lung function
Abnormal lung function: FVC <80% predicted or diffusion capacity of the lung for carbon monoxide (D_{LCO}) <40% predicted
Any dyspnea or functional limitation attributable to lung disease
Any oxygen requirement, even if only during exertion
For inflammatory ILD, failure to improve dyspnea, oxygen requirement, and/or lung function after a clinically indicated trial of medical therapy
Transplantation guidelines
Decline in FVC ≥10% during 6 months of follow-up
Decline in D_{LCO} ≥15% during 6 months of follow-up
Desaturation to <88% or distance <250 m on 6-minute walk test or >50 m decline in 6-minute walk distance over a 6-month period
Pulmonary hypertension on right heart catheterization or 2-dimensional echocardiography
Hospitalization because of respiratory decline, pneumothorax, or acute exacerbation

UIP, usual interstitial pneumonitis; NSIP, non-specific interstitial pneumonia; FVC, forced vital capacity; ILD, interstitial lung disease.

and rheumatoid arthritis.

IPF and fibrotic non-specific interstitial pneumonia (NSIP)

Prognostic factors

Over the last years, a great interest has been developed in IPF, since the introduction of antifibrotic drugs, which was the first ever treatment for this disease that seemed to slow its progression and to impact on mortality. Several guidelines for diagnosis of IPF have been released, mainly based on radiologic criteria, with an UIP pattern being associated with a worse prognosis (49,50). It became clear that the prognosis of IPF is indeed very bad, with a 50% survival of 2–3 years after the diagnosis (51,52), which is much worse compared to other DPLD. Several disease characteristics that have an impact on prognosis have been identified such as the % pred., FVC at diagnosis, the rate of decline of FVC over 6 months, the diffusing capacity for CO and the occurrence of exacerbations (53).

Indeed, Nathan *et al.* showed that patients divided by FVC (mild, ≥70%; moderate, 55% to 69%; and severe, <55%) had correspondingly worse median survival of 55.6, 38.7, and 27.4 months, respectively (54). Also, longitudinal change in FVC has been demonstrated to be a risk factor for increased mortality in multiple studies, and even marginal changes in FVC (5% to 10%) over a 6-month were associated with a higher mortality than in patients with stable disease (53,55). Furthermore, also D_{LCO} was

significantly associated with survival on multivariate analysis, with the hazard of death increasing by 4% for every 1% decrease in D_{LCO} (56). In longitudinal analysis, a greater than 20% decline in D_{LCO} at 1 year was found to be significantly correlated with mortality (57). Acute exacerbations of IPF precede IPF mortality in up to 50% of the patients and are associated with a high in-hospital mortality (up to 50%) and a median survival afterwards of only 3–5 months (58,59). Pulmonary hypertension is also regarded as a risk factor for the outcome in patients with IPF with 5-year survival declining as mean pulmonary arterial pressure rose above 17 mmHg (60).

As a consequence, timely referral of these patients is very much needed. This is even more of interest as an important survival benefit after transplantation for IPF has been demonstrated in several series (53,61). Criteria for referral and listing are summarized in *Table 3*.

For other DPLD (for instance LAM, ILD associated with collagen vascular diseases), no clear criteria have been issued so far, but if the pulmonary disease is severe enough to warrant consideration of lung transplantation and the lung disease has not responded to appropriate treatment and there are no extrapulmonary contraindications to transplantation, it is reasonable to use similar guidelines to those proposed for IPF (7). For sarcoidosis, patients can be referred for transplantation if they are at least in New York Heart Association class III, and should be transplanted if they meet one of these further criteria:

Table 4 Referral and transplantation guidelines in CF and non-CF bronchiectasis (6)

Referral guidelines
FEV$_1$ <30% pred. or a patient with a rapidly falling FEV$_1$ despite optimal therapy (particularly in a female patient), infected with NTM or B cepacia complex (see general indications/contra-indications) and/or with diabetes
A 6-minute walk distance <400 m
Development of pulmonary hypertension in the absence of a hypoxic exacerbation (a systolic PAP >35 mmHg on echocardiography or mean PAP >25 mmHg measured by right heart catheterization)
Clinical decline characterized by increasing frequency of exacerbations associated with one of the following conditions
An episode of acute respiratory failure requiring non-invasive ventilation
Increasing antibiotic resistance and poor clinical recovery from exacerbations
Worsening nutritional status despite supplementation
Pneumothorax
Life-threatening hemoptysis despite bronchial embolization
Transplantation guidelines
Chronic respiratory failure, either hypoxia alone (PaO$_2$ <8 kPa or <60 mmHg) and/or hypercapnia (PaCO$_2$ >6.6 kPa or >50 mmHg)
On long-term non-invasive ventilation
Increasing pulmonary hypertension (as defined above)
Frequent hospitalization
Rapid lung function decline (especially in females)
World Health Organization functional class IV

NTM, non-tuberculous mycobacterial; PAP, pulmonary arterial pressure.

(I)   Hypoxemia at rest;
(II)   Pulmonary hypertension;
(III)   Elevated right atrial pressure >15 mmHg.

A specific caution for IPF patients on antifibrotic treatment (pirfenidone of nintedanib) needs to be mentioned: despite these drugs have an impact on disease progression, on exacerbation rates and on survival (62-65), this treatment may not delay referral of IPF patients for consideration of lung transplantation, but it may buy time on the waiting list as in some countries wait list mortality for IPF is still very high, up to >30% (66). On the other hand, treatment with these drugs has no adverse outcome after transplantation and does not need to be stopped when patients are listed (67).

CF and non-CF bronchiectasis

CF is one of the major indications for lung transplantation; accounting for about 15% of all transplants in 2014 (1). CF mostly affects younger patients compared to other diagnosis necessitating lung transplantation. The survival is therefore reported to be consistently higher, with a mean 5-year survival of 62.5%, compared to 53.8% for COPD and 48.5% for idiopathic interstitial diseases (1). In our own experience, the actuarial 5- and 10-year survival in a cohort of CF patients transplanted between 2005 and 2015 (n=81) was 90% and 86% respectively (68).

Non-CF bronchiectasis may have different causes, and in a recent study, among the 1,258 patients enrolled, an etiology of bronchiectasis was determined in 60%, including post-infective (20%), chronic obstructive pulmonary disease related (15%), connective tissue disease related (10%), immunodeficiency related (5.8%), and asthma related (3.3%). In 40% of patients, there was no specific cause identified (idiopathic bronchiectasis) (68). Although mostly older than CF patients (mean age 67 years, 58–75 years) (69), the referral and transplantation criteria are comparable to CF patients (*Table 4*).

These referral and transplantation criteria are mainly based on the publication by Kerem *et al.* (70). These authors analyzed mortality predictors in 673 CF patients, and clearly demonstrated that the prognosis of CF patients is

related to FEV$_1$ (<30% pred.), a PaO$_2$ <55 mmHg, a PaCO$_2$ >50 mmHg and the BMI. They also concluded that female patients and younger patients had a worse prognosis (70). Since the publication of Kerem *et al.*, prognosis of CF patients has surely improved, but nevertheless, these criteria are still useable when evaluating a CF patient for possible transplantation.

Several predictive models for 5-year survival have been published so far, and although mostly valid in the tested population, they proved to be wrong in a control population. This may have to do with local treatment options and habits.

Special considerations in CF patients

Microbial and fungal colonization of the airways is abundant in CF patients. As already mentioned in the general contra-indications section of this chapter, colonization with B. Cepacia and especially *Burkholderia cepacia* complex (BCC) or *Burkholderia cenocepacia* may be a contra-indication for transplantation in some centers. Indeed, the Newcastle group recently published their experience with lung transplantation in CF patients with BCC: of 216 CF patients transplanted, 22 had BCC of whom 12 *Burkholderia cenocepacia*. Nine *Burkholderia cenocepacia*-infected recipients died within the first year, and 8 sepsis were considered to be the cause of death. These results lead this group to further decline patients with pre-transplant colonization with *Burkholderia cenocepacia* (71), whereas this may be an acceptable risk for others. Acceptance of such patients for lung transplantation will thus depend on experience of the team and initial outcomes with such patients.

On the other hand, CF patients colonized with *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* have similar post-transplant survival as compared to other CF patients, irrespective of their antibiotic susceptibility patterns. The presence of these organisms should not preclude lung transplantation (72).

Colonization of the airways with *Mycobacterium abscessus* also leads to conflicting results, with some centers having good outcome whereas others do regard this colonization as a contra-indication (see section on general contra-indications) (21,22).

Colonization with *Scedosporium apiospermum* may have an identical impact, with some centers declining such patients and others accepting them, provided they are actively treated with azole derivatives and receive lifelong azole treatment after transplantation when they get colonized (73).

Some CF patients have overt liver disease, evolving to cirrhosis, which is recognized as an independent risk factor for death or lung transplantation. In that case, a combined liver and lung transplantation procedure can be performed, with good outcome. We recently reported our own experience with 11 combined lung and liver transplantations, of which five patients had CF. The 5-year patient survival was 90% (8).

Pulmonary arterial hypertension (PAH)

PAH, and more specifically idiopathic PAH (iPAH) and chronic thromboembolic PAH remain a valid indication for lung transplantation (1,6). The number of lung transplantations for these conditions has gradually decreased, given the better treatment options that have become available over the last 10 years. Nowadays, <3% of all indications for lung transplantation are performed in iPAH and <2% in non-iPAH patients (1). If patients with PAH fail their usual (triple) treatment regimen (prostanoids, endothelin receptor antagonists, and phosphodiesterase inhibitors), they might need a lung transplant. For most of these patients who can become very debilitated in a short time, the transplant window is often very short, and sometimes, they will need a rather urgent transplant. In that case, the LAS may help to prioritize patients with PAH for transplantation, as has recently been shown in Germany. Indeed, patients with PAH had a mean LAS score of 53, which was even higher than for the IPF patients (74).

Several risk factors for worse outcome have been identified in PAH, such as the etiology of the PAH, male sex, older age, worse functional class, 6-minute walking distance, hemodynamic parameters, BNP and NT-proBNP values, etc. (75,76). These and other risk factors are used in the REVEAL scoring system, which is a quantitative equation for predicting survival and was prospectively validated in a cohort of newly diagnosed PAH patients from the REVEAL registry (77). Referral and transplantation guidelines are summarized in *Table 5*.

Conclusions

Guidelines for referral and transplantation are only guidelines and not an exact science. These guidelines give an idea when to think about lung transplantation and serve to refer a patient in time for transplantation. Of course, difficult situations remain and will not be solved by these guidelines. In general, we always ask our referring physicians

Table 5 Referral and transplantation guidelines in PAH (6)

Referral guidelines
NYHA functional class III or IV symptoms during escalating therapy
Rapidly progressive disease (assuming weight and rehabilitation concerns not present)
Use of parenteral targeted PAH therapy regardless of symptoms or NYHA functional class
Known or suspected pulmonary veno-occlusive disease or pulmonary capillary hemangiomatosis
Transplantation guidelines
NYHA functional class III or IV despite a trial of at least 3 months of combination therapy including prostanoids
Cardiac index of O_2 liters/min/m^2
Mean right atrial pressure of >15 mmHg
6-minute walk test of <350 m

Development of significant hemoptysis, pericardial effusion, or signs of progressive right heart failure (renal insufficiency, increasing bilirubin, brain natriuretic peptide, or recurrent ascites).

to discuss every potential lung transplant candidate with the transplant center before referral. Also, a lot of decisions will depend upon the local waiting list, waiting times and outcomes of lung transplantation in a specific center. This is especially the case in so called difficult indications for lung transplantation (colonization with highly resistant bacteria, as discussed under CF, patients with scleroderma and severe reflux/dysmotility of the esophagus, etc.), which always need to be discussed with the transplant center before taking any decision.

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Footnote

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References

1. Yusen RD, Edwards LB, Dipchand AI, et al. Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.
2. Maurer JR, Frost AE, Estenne M, et al. International guidelines for the selection of lung transplant candidates. The International Society for Heart and Lung Transplantation, the American Thoracic Society, the American Society of Transplant Physicians, the European Respiratory Society. J Heart Lung Transplant 1998;17:703-9.
3. Maurer JR, Frost AE, Estenne M, et al. International guidelines for the selection of lung transplant candidates. The International Society for Heart and Lung Transplantation, the American Thoracic Society, the American Society of Transplant Physicians, the European Respiratory Society. Heart Lung 1998;27:223-9.
4. Maurer JR, Frost AE, Estenne M, et al. International guidelines for the selection of lung transplant candidates. The International Society for Heart and Lung Transplantation, the American Thoracic Society, the American Society of Transplant Physicians, the European Respiratory Society. Transplantation 1998;66:951-6.
5. Orens JB, Estenne M, Arcasoy S, et al. International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2006;25:745-55.
6. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.
7. Barshes NR, DiBardino DJ, McKenzie ED, et al. Combined lung and liver transplantation: The United States experience. Transplantation 2005;80:1161-7.
8. Ceulemans LJ, Strypstein S, Neyrinck A, et al. Combined liver-thoracic transplantation: single-center experience

with introduction of the 'Liver-first' principle. Transpl Int 2016;29:715-26.

9. Ceulemans LJ, Monbaliu D, Verslype C, et al. Combined liver and lung transplantation with extended normothermic lung preservation in a patient with end-stage emphysema complicated by drug-induced acute liver failure. Am J Transplant 2014;14:2412-6.

10. Bertani A, Grossi P, Vitulo P, et al. Successful lung transplantation in an HIV- and HBV-positive patient with cystic fibrosis. Am J Transplant 2009;9:2190-6.

11. Fong TL, Cho YW, Hou L, et al. Outcomes after lung transplantation and practices of lung transplant programs in the United States regarding hepatitis C seropositive recipients. Transplantation 2011;91:1293-6.

12. Dobbels F, De Bleser L, Berben L, et al. Efficacy of a medication adherence enhancing intervention in transplantation: The MAESTRO-Tx trial. J Heart Lung Transplant 2017;36:499-508.

13. Ruttens D, Verleden SE, Goeminne PC, et al. Smoking resumption after lung transplantation: standardised screening and importance for long-term outcome. Eur Respir J 2014;43:300-3.

14. Yusen RD, Christie JD, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Adult Lung and Heart-Lung Transplant Report--2013; focus theme: age. J Heart Lung Transplant 2013;32:965-78.

15. Plöchl W, Pezawas L, Artemiou O, et al. Nutritional status, ICU duration and ICU mortality in lung transplant recipients. Intensive Care Med 1996;22:1179-85.

16. Lederer DJ, Wilt JS, D'Ovidio F, et al. Obesity and underweight are associated with an increased risk of death after lung transplantation. Am J Respir Crit Care Med 2009;180:887-95.

17. Ruttens D, Verleden SE, Vandermeulen E, et al. Body mass index in lung transplant candidates: a contra-indication to transplant or not? Transplant Proc 2014;46:1506-10.

18. Hirt SW, Rahimi A, Möller F, et al. Does previous thoracic surgery increase perioperative risk in lung transplantation? Transplant Proc 2001;33:3572-3.

19. Hayanga JW, Lira A, Aboagye JK, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation: what lessons might we learn from volume and expertise? Interact Cardiovasc Thorac Surg 2016;22:406-10.

20. Biscotti M, Gannon WD, Agerstrand C, et al. Awake Extracorporeal Membrane Oxygenation as Bridge to Lung Transplantation: A 9-Year Experience. Ann Thorac Surg 2017;104:412-9.

21. Lobo LJ, Chang LC, Esther CR Jr, et al. Lung transplant outcomes in cystic fibrosis patients with pre-operative Mycobacterium abscessus respiratory infections. Clin Transplant 2013;27:523-9.

22. Smibert O, Snell GI, Bills H, et al. Mycobacterium abscessus Complex - a Particular Challenge in the Setting of Lung Transplantation. Expert Rev Anti Infect Ther 2016;14:325-33.

23. Olland A, Falcoz PE, Kessler R, et al. Should cystic fibrosis patients infected with Burkholderia cepacia complex be listed for lung transplantation? Interact Cardiovasc Thorac Surg 2011;13:631-4.

24. Quon BS, Reid JD, Wong P, et al. Burkholderia gladioli - a predictor of poor outcome in cystic fibrosis patients who receive lung transplants? A case of locally invasive rhinosinusitis and persistent bacteremia in a 36-year-old lung transplant recipient with cystic fibrosis. Can Respir J 2011;18:e64-5.

25. Kern RM, Seethamraju H, Blanc PD, et al. The feasibility of lung transplantation in HIV-seropositive patients. Ann Am Thorac Soc 2014;11:882-9.

26. Sweet MP, Herbella FA, Leard L, et al. The prevalence of distal and proximal gastroesophageal reflux in patients awaiting lung transplantation. Ann Surg 2006;244:491-7.

27. Palmer SM, Miralles AP, Howell DN, et al. Gastroesophageal reflux as a reversible cause of allograft dysfunction after lung transplantation. Chest 2000;118:1214-7.

28. Young LR, Hadjiliadis D, Davis RD, et al. Lung transplantation exacerbates gastroesophageal reflux disease. Chest 2003;124:1689-93.

29. Shah N, Force SD, Mitchell PO, et al. Gastroesophageal reflux disease is associated with an increased rate of acute rejection in lung transplant allografts. Transplant Proc 2010;42:2702-6.

30. Blondeau K, Mertens V, Vanaudenaerde BA, et al. Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. Eur Respir J 2008;31:707-13.

31. Meyer KC, Raghu G, Verleden GM, et al. ISHLT/ATS/ERS BOS Task Force Committee. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J 2014;44:1479-503.

32. Hartwig MG, Anderson DJ, Onaitis MW, et al. Fundoplication after lung transplantation prevents the allograft dysfunction associated with reflux. Ann Thorac Surg 2011;92:462-8.

40

Verleden et al. Recipient selection for lung transplantation

33. Gulack BC, Meza JM, Lin SS, et al. Reflux and allograft dysfunction: is there a connection? Thorac Surg Clin 2015;25:97-105.

34. Verleden GM, Vos R, Vanaudenaerde B, et al. Current views on chronic rejection after lung transplantation. Transpl Int 2015;28:1131-9.

35. Miele CH, Schwab K, Saggar R, et al. Lung Transplant Outcomes in Systemic Sclerosis with Significant Esophageal Dysfunction. A Comprehensive Single-Center Experience. Ann Am Thorac Soc 2016;13:793-802.

36. Egan TM, Edwards LB. Effect of the lung allocation score on lung transplantation in the United States. J Heart Lung Transplant 2016;35:433-9.

37. Celli BR, Cote CG, Marin JM, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med 2004;350:1005-12.

38. Marchand E. The BODE index as a tool to predict survival in COPD lung transplant candidates. Eur Respir J 2010;36:1494-5.

39. Eskander A, Waddell TK, Faughnan ME, et al. BODE index and quality of life in advanced chronic obstructive pulmonary disease before and after lung transplantation. J Heart Lung Transplant 2011;30:1334-41.

40. Lane CR, Tonelli AR. Lung transplantation in chronic obstructive pulmonary disease: patient selection and special considerations. Int J Chron Obstruct Pulmon Dis 2015;10:2137-46.

41. Lahzami S, Bridevaux PO, Soccal PM, et al. Survival impact of lung transplantation for COPD. Eur Respir J 2010;36:74-80.

42. Soler-Cataluña JJ, Martínez-García MA, Román Sánchez P, et al. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. Thorax 2005;60:925-31.

43. Soler-Cataluña JJ, Martínez-García MA, Sánchez LS, et al. Severe exacerbations and BODE index: two independent risk factors for death in male COPD patients. Respir Med 2009;103:692-9.

44. Connors AF Jr, Dawson NV, Thomas C, et al. Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (Study to Understand Prognoses and Prefer ences for Outcomes and Risks of Treatments). Am J Respir Crit Care Med 1996;154:959-67.

45. Tokgoz Akyil F, Gunen H, Agca M, et al. Patient Outcome after Chronic Obstructive Pulmonary Disease Exacerbations Requiring Non-invasive Ventilation during Hospitalization. Arch Bronconeumol 2016;52:470-6.

46. National Emphysema Treatment Trial Research Group, Fishman A, Fessler H, et al. Patients at high risk of death after lung-volume-reduction surgery. N Engl J Med 2001;345:1075-83.

47. Klooster K, Ten Hacken NH, Hartman JE, et al. Endobronchial valves for emphysema without interlobar collateral ventilation. N Engl J Med 2015;373:2325-35.

48. Klooster K, Hartman JE, Ten Hacken NH, et al. One-Year Follow-Up after Endobronchial Valve Treatment in Patients with Emphysema without Collateral Ventilation Treated in the STELVIO Trial. Respiration 2017;93:112-21.

49. Raghu G, Collard HR, Egan JJ, et al. ATS/ERS/JRS/ ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788-824.

50. Wilson KC, Raghu G. The 2015 guidelines for idiopathic pulmonary fibrosis: an important chapter in the evolution of the management of patients with IPF. Eur Respir J 2015;46:883-6.

51. Bjoraker JA, Ryu JH, Edwin MK, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1998;157:199-203.

52. Thomeer MJ, Vansteenkiste J, Verbeken EK, et al. Interstitial lung diseases: Characteristics at diagnosis and mortality risk assessment. Respir Med 2004;98:567-73.

53. Latsi PI, du Bois RM, Nicholson AG, et al. Fibrotic idiopathic interstitial pneumonia: The prognostic value of longitudinal functional trends. Am J Respir Crit Care Med 2003;168:531-7.

54. Nathan SD, Shlobin OA, Weir N, et al. Long-term course and prognosis of idiopathic pulmonary fibrosis in the new millennium. Chest 2011;140:221-9.

55. Zappala CJ, Latsi PI, Nicholson AG, et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. Eur Respir J 2010;35:830-6.

56. Mogulkoc N, Brutsche MH, Bishop PW, et al. Pulmonary function in idiopathic pulmonary fibrosis and referral for lung transplantation. Am J Respir Crit Care Med 2001;164:103-8.

57. Hanson D, Winterbauer RH, Kirtland SH, et al. Changes in pulmonary function test results after 1 year of therapy as predictors of survival in patients with idiopathic pulmonary fibrosis. Chest 1995;108:305-10.

58. Simon-Blancal V, Freynet O, Nunes H, et al. Acute exacerbation of idiopathic pulmonary fibrosis: outcome and prognostic factors. Respiration 2012;83:28-35.

59. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. Am J Respir Crit Care Med 2016;194:265-75.

60. Nadrous HF, Pellikka PA, Krowka MJ, et al. The impact of pulmonary hypertension on survival in patients with idiopathic pulmonary fibrosis. Chest 2005;128:616S-617S.

61. Thabut G, Mal H, Castier Y, et al. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. J Thorac Cardiovasc Surg 2003;126:469-75.

62. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet 2011;377:1760-9.

63. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. Eur Respir J 2016;47:243-53.

64. Richeldi L, Costabel U, Selman M, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med 2011;365:1079-87.

65. Richeldi L, du Bois RM, Raghu G, et al. INPULSIS Trial Investigators. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med 2014;370:2071-82.

66. ten Klooster L, Nossent GD, Kwakkel-van Erp JM, et al. Ten-Year Survival in Patients with Idiopathic Pulmonary Fibrosis After Lung Transplantation. Lung 2015;193:919-26.

67. Delanote I, Wuyts WA, Yserbyt J, et al. Safety and efficacy of bridging to lung transplantation with antifibrotic drugs in idiopathic pulmonary fibrosis: a case series. BMC Pulm Med 2016;16:156.

68. Vos R, Verleden GM, Dupont LJ. Long-term survival after lung transplantation among cystic fibrosis patients: Moving away from mere palliation. J Heart Lung Transplant 2016;35:837-40.

69. Lonni S, Chalmers JD, Goeminne PC, et al. Etiology of Non-Cystic Fibrosis Bronchiectasis in Adults and Its Correlation to Disease Severity. Ann Am Thorac Soc 2015;12:1764-70.

70. Kerem E, Reisman J, Corey M, et al. Prediction of mortality in patients with cystic fibrosis. N Engl J Med 1992;326:1187-91.

71. De Soyza A, Meachery G, Hester KL, et al. Lung transplantation for patients with cystic fibrosis and Burkholderia cepacia complex infection: a single-center experience. J Heart Lung Transplant 2010;29:1395-404.

72. Lobo LJ, Tulu Z, Aris RM, et al. Pan-Resistant Achromobacter xylosoxidans and Stenotrophomonas maltophilia Infection in Cystic Fibrosis Does Not Reduce Survival After Lung Transplantation. Transplantation 2015;99:2196-202.

73. Parize P, Boussaud V, Poinsignon V, et al. Clinical outcome of cystic fibrosis patients colonized by Scedosporium species following lung transplantation: A single-center 15-year experience. Transpl Infect Dis 2017. [Epub ahead of print].

74. Gottlieb J, Greer M, Sommerwerck U, et al. Introduction of the lung allocation score in Germany. Am J Transplant 2014;14:1318-27.

75. Humbert M, Sitbon O, Yaici A, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. Eur Respir J 2010;36:549-55.

76. Raina A, Humbert M. Risk assessment in pulmonary arterial hypertension. Eur Respir Rev 2016;25:390-8.

77. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation 2010;122:164-72.

Lung allocation

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Abstract: Shortage of donor lungs in most western countries and broadening of indications for lung transplantation (LTx) has led increased waiting list mortality in the past. Usually donor lungs and recipients are matched by size as measured by total lung capacity and blood type in first order. In some countries regional allocation comes first, in other countries a national wait list exists and some nations are organized in supranational allocation systems. Organ distribution should respect the ethical principles of equity, justice, beneficence and utility. Generally, top priority on the list should be given to patients with the least amount of time to live but outcome is an important factor to consider to avoid futile transplantations. Installation of an urgency status will decrease mortality of the sickest candidates on the waitlist unless the proportion of patients on urgency status will be too high. Urgency can be determined by clinical judgment (so called center decision), an audit process or objectively by a score system. Among the 3,500 transplants performed worldwide annually, approximately 60% are allocated by lung allocation score (LAS) (US, Germany, the Netherlands). With the LAS a model for survival prediction after lung transplantation and wait list survival probability was created. Clinical experience in the US since 2005 and in Germany since 2011 favourable reports regarding effects on waiting list outflow, transplant activity and outcomes have been published. Future perspectives will focus on broader geographic sharing, updating and further development of the LAS.

Keywords: Lung transplantation; waiting list; lung allocation score (LAS); organ procurement; organ donation

Introduction

Currently, approximately 3,500 transplants are performed worldwide annually (1). Eligible patients are put on the waiting list at their local transplant centre. Unfortunately, a shortage of donor organs leads to considerable numbers of patients dying on the waiting list for lung transplantation (LTx) before suitable organs become available. Lung transplantation is nowadays an accepted therapy for end-stage thoracic disease, but major problems remain to be addressed. Transplantation is has a costly and risky therapy. Demand for donor lungs exceeds the supply by far. The discrepancy between supply of organs and demand for them as life-saving therapies has resulted in scrutiny of organ distribution policies, and raises ethical questions. In designing an allocation algorithm, a number of ethical principles should be considered including the four basic ethical principles:

(I)   Patient autonomy: a patient has the right to choose/refuse treatment;
(II) Beneficence: practitioner should act in the best interest of the patient;
(III) Non-maleficence: an obligation to not intentionally inflict harm to the patient;
(IV) Justice: concerns the distribution of scarce health resources ("Who gets what?").

Efficient donor organ allocation remains crucial in optimizing donor use, to reduce waitlist mortality and to improve transplant outcomes.

In some countries, there is national wait list and some countries are organized in supranational allocation systems (e.g., Eurotransplant). Available donor lungs will be assigned according to predetermined criteria. Usually lungs

Table 1 Comparison of different allocation models

Principle	Center decision	Waiting time plus urgency	Allocation score
Equity	(+)	+	++
Justice	(+)	+	(+)
Beneficence	(+)	(+)	++
Utility	(+)	(+)	++
Survival	(+)	+	++
Quality of life	(+)	–	–
Countries	UK, Italy, Belgium	Switzerland, France	US, Germany, the Netherlands

(+), variably influenced; +, influenced; ++, strongly influenced; –, not influenced.

are matched by size (total lung capacity) and blood type in first order. In case of several suitable candidates for a given organ offer, organs are distributed according further pre-specified rules. The entire process of organ distribution is called "organ allocation".

Allocation criteria after blood type and size matching may be based on clinical judgment (so called center decision), urgency (e.g., by audit process, individual decision, or objectively by a score system), or on waiting time, or a combination of several of these criteria. Currently, rules guiding allocation in most countries are based on urgency and transplant benefit, with survival benefit being the accepted primary goal. The ideal time for the transplant is not easy to determine and depends on the individual course of the underlying illness. Many pulmonary diseases experience a relatively slowly progressive course while other will develop a sudden acceleration with rapid deterioration of the patient's condition. Within a system of waiting time based allocation only up to 30% of patients will die before an organ becomes available (2). Installation of an urgency status will decrease mortality of critically ill candidates unless the proportion of patients on urgency status will be too high (3).

Most people agree that top priority should be given to patients with the least amount of time to live ('Rule of Rescue') and outcome is rated second by the majority. Existing registry data are often used to assist individual assessment of urgency and transplant benefit in conjunction with clinical judgment. Historically, lung allocation, in the US and in the Eurotransplant region, was mainly based on waiting time. In some European countries there is a national urgency list (France, Switzerland) and some European countries allocate donor lungs according center decision (UK). More than 60% of the worldwide lung transplant activity is allocated by

the lung allocation score (LAS). Waiting time-based lung allocation and center-based allocation has been reported to be associated with high wait list mortality (2,4).

The pros and cons of the three most commonly used allocation models (center decision, waiting time plus urgency and an allocation score) are displayed in *Table 1*.

The LAS

The LAS is a numerical value used to assign relative priority in distributing donated lungs. The LAS evaluates several parameters of patient health to direct organ donation toward patients obtaining greatest benefit from lung transplantation (5).

More than a decade ago, the US Department of Health and Human Services issued the "Final Rule", intended to ensure that organs were allocated "based on medical criteria, not accidents of geography". In 1998, the Department of Health and Human Services of the US suggested that waiting time-based allocation should be replaced by medical urgency in the absence of unsuccessful transplants. Urgency was classified as more important than the prospect of success. A working group was set up to develop a corresponding system for lung allocation. Essential ethical aspects in the development were equality of all patients (blood group, ethnicity), justice (each patient is judged strictly according to objective criteria), benefit (principle: the benefit must outweigh the potential damage), and usefulness (benefit of a scarce resource).

To develop a statistical model, 3,104 American Lung Transplant-data recorded in the US Register OPTN were analysed from candidates of the years 1997 and 1998. Eighty percent of all listings were made according to four diagnostic groups [lung emphysema/chronic obstructive lung disease (COPD) including alpha1-

antitrypsin deficiency (n=1,461), cystic fibrosis (n=708), idiopathic pulmonary fibrosis (n=608) and idiopathic pulmonary arterial hypertension (n=327)].

Because of the relatively small number of patients with idiopathic pulmonary arterial hypertension, this group was enriched by patients from the years 1995 and 1996 so that in the end, 636 of these patients were available for evaluation. Approximately 30 parameters of lung transplant candidates were recorded in the US at that time on the wait list. These included age, height, weight, body mass index (BMI), pulmonary arterial pressure (PAP), cardiac index (CI), pulmonary capillary wedge pressure (PCWP), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), functional status, etc.

In the four diagnosis groups mentioned above, multivariate Cox analysis was carried out, which resulted in disease-specific factors which had a significant influence on the wait list mortality.

The remaining 20% of the patients who were initially not covered by these groups were assigned to the four main groups on the basis of clinically similar symptoms and courses. This assignment was statistically verified by comparing the survival of the respective patients with the calculated survival of the group.

In addition, the results were adjusted by combining the parameters of all four groups into a total model. These factors were confirmed in their prognostic statement by separate analysis of the four disease categories. With the overall analysis of all patients, both the probable survival within a year on the wait list, as well as the 1-year survival after lung transplantation could be calculated using biometric and clinical data. Similarly, prognostic factors for 1-year survival after transplantation had been calculated in a multivariate regressive Cox analysis. The restriction to 1 year was made, since after this time the influence of factors, which determines the immediate success of transplantation, hardly could have any effect on the result.

The models for survival after lung transplantation and wait list survival probability were combined to form a model in which the actual benefit of the transplant was calculated as the difference between transplant survival and wait list survival. Double weighing of wait list survival corresponded to the original intention to take the urgency more into consideration than the success prospect. The LAS takes into account the estimated survival benefit offered by LTx by 1 year after surgery and medical urgency. Parameters included in the model are displayed in *Table 2*. LAS can be appointed a value between 0 and 100 and according to

this model rates the estimated survival advantage by LTx to 1 year. The aim of the system is to direct organs to recipients who are predicted to have the greatest potential transplantation survival benefit.

The LAS system was introduced in the US in May 2005 and has also been adopted in Germany in December 2011 for LTx candidates age 12 and older and in the Netherlands in April 2014. In the years following implementation in the US and Germany, numerous mainly favourable reports regarding effects on waiting list outflow, transplant activity and outcomes have been published. In the US and Germany constant reduction of mortality on the wait list was observed translated to approximately 8 lives saved on the waiting lists per 100 lung transplants performed (3,6).

Since its introduction as a tool for donor lung allocation in the US in 2005, the number of LTx for US CF patients has increased by 25%. Of note, 70% of wait-listed CF patients were transplanted after a waiting period of 1 year decreasing the 1-year waiting-list mortality from 15% to 10% (7).

Pulmonary hypertension accounts for approximately 5% of all lung transplant activity, with improvements in medical therapy leading to global declines in waiting list registrations and transplant activity. Existing US data on LAS performance for PH patients have revealed somewhat conflicting findings (8,9). Initial reports failed to demonstrate improved waiting list mortality among candidates with pulmonary hypertension, however, more recent analysis involving larger cohorts have contested this. The German data, whilst also limited by small sample size supports this latter report, showing clear reductions in waiting list mortality (3). Composition of transplant recipients changed, with fewer patients with obstructive lung diseases (e.g., COPD) and more recipients with restrictive lung diseases (e.g., idiopathic pulmonary fibrosis). Transplantation under invasive mechanical respiratory support increased in Germany from 9% to 13% (3).

In February 2015 a new LAS model was introduced in the US after the Thoracic Organ Transplantation Committee proposed a revision to the LAS system. This revision includes modifications to the covariates in the waiting list and post-transplant survival models, coefficients of the covariates, and baseline waiting list and post-transplant survival rates used in the LAS calculation. New parameters like increase in creatinine and bilirubin, central venous pressure (CVP), CI (if less than 2 $L/min/m^2$), 6-min-walk distance (if it is less than 1,200 feet), oxygen needed at rest were included in the 2015 LAS model, while others were abandoned. Results of performance of the new model are not

Table 2 Parameters of the 2010 LAS model

Age (years)
Height (cm)
Weight (kg)
Diagnosis of lung disease
Functional status (without support, mild support, full support)
Diabetes status (unknown, insulin-dependent, no diabetes, non-insulin-dependent)
Mechanical ventilation [none, continuous positive airway pressure (CPAP), bilevel Positive airway pressure (BiPAP), continuous invasive, intermittent invasive]
Oxygen treatment (none, at rest, only at night, only under stress)
Oxygen requirement at rest (L/min or %)
Forced vital capacity (% predicted)
Systolic pulmonary arterial pressure (mmHg)
Mean pulmonary arterial pressure (mmHg)
Mean pulmonary capillary closing pressure (mmHg)
Current carbon dioxide partial pressure (mmHg or kPa)
Rise in carbon dioxide partial pressure (%) in relation to minimal carbon dioxide partial pressure
6-min walking test (m)
Serum creatinine (µmol/L or mg/dL)

LAS, lung allocation score.

yet published. Germany has decided to continue with the 2010 model in 2017.

The LAS can be computed online (2010 model: http://www.eurotransplant.org/cms/index.php?page=las_calculator and 2015 model: https://optn.transplant.hrsa.gov/resources/allocation-calculators/las-calculator/). In the US, organ distribution is made locally first, while in Germany distribution is nationally in first step.

Broader geographic sharing may increase travel costs and ischemic time, but a more appropriate recipient might be identified closer to the donor in a neighboring donor service area. It could be demonstrated recently that 53% of the lungs in the US were transplanted locally (within 58 donor service areas). For each local allocation, a median of 6 recipients in a larger region (nationwide there are 11 regions) had higher LAS values in this retrospective analysis (10). There are practical limitations to transportation of donor lungs because increasing ischemic time and graft dysfunction is related to increasing donor age. Any lung organ distribution system needs to take into account the large size of this country, and a 'national´ list is probably impractical for large countries.

The transplantation center is responsible for the correct procurement and regular update of LAS (usually in intervals of 3 months, in critical patients every 2 weeks). It should be noted that LAS is not a suitable tool for identifying candidates. This must be performed via individual patient assessment by the transplant team.

No allocation system can eliminate death on the waiting list. Therefore distribution of donor lungs as a precious resource should be made wisely and fairly.

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Footnote

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References

1. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung

Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant 2015;34:1264-77.

2. De Meester J, Smits JM, Persijn GG, et al. Listing for lung transplantation: life expectancy and transplant effect, stratified by type of end-stage lung disease, the Eurotransplant experience. J Heart Lung Transplant 2001;20:518-24.

3. Gottlieb J, Smits J, Schramm R, et al. Lung Transplantation in Germany Since the Introduction of the Lung Allocation Score. Dtsch Arztebl Int 2017;114:179-85.

4. Mackay LS, Anderson RL, Parry G, et al. Pulmonary fibrosis: rate of disease progression as a trigger for referral for lung transplantation. Thorax 2007;62:1069-73.

5. Egan TM, Murray S, Bustami RT, et al. Development of the new lung allocation system in the United States. Am J Transplant 2006;6:1212-27.

6. Egan TM, Edwards LB. Effect of the lung allocation score on lung transplantation in the United States. J Heart Lung Transplant 2016;35:433-9.

7. Thabut G, Christie JD, Mal H, et al. Survival benefit of lung transplant for cystic fibrosis since lung allocation score implementation. Am J Respir Crit Care Med 2013;187:1335-40.

8. Chen H, Shiboski SC, Golden JA, et al. Impact of the lung allocation score on lung transplantation for pulmonary arterial hypertension. Am J Respir Crit Care Med 2009;180:468-74.

9. Schaffer JM, Singh SK, Joyce DL, et al. Transplantation for idiopathic pulmonary arterial hypertension: improvement in the lung allocation score era. Circulation 2013;127:2503-13.

10. Russo MJ, Meltzer D, Merlo A, et al. Local allocation of lung donors results in transplanting lungs in lower priority transplant recipients. Ann Thorac Surg 2013;95:1231-4; discussion 1234-5.

Bridging to lung transplantation with extracorporeal circulatory support: when or when not?

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Abstract: Patients with end-stage lung disease who are candidates for lung transplantation may acutely decompensate before a donor organ becomes available. In this scenario, extracorporeal life support (ECLS) may be considered as a bridge to transplant or as a bridge to decision. In the current chapter, we review the indications, techniques, and outcomes for bridging to lung transplantation with ECLS.

Keywords: Lung transplantation; extracorporeal membrane oxygenation (ECMO); bridging; extracorporeal life support (ECLS)

Introduction

Lung transplantation is a viable treatment option for patients with end-stage lung disease. This is especially true for patients who present with a rapid decline in respiratory status, requiring advanced airway support. These patients have a high rate of in-hospital mortality, and their candidacy for lung transplantation should be urgently assessed (1-3). Unfortunately, donor lungs are scarce, so bridging strategies may be necessary for survival.

The implementation of the Lung Allocation Score and Eurotransplant high-urgency status has significantly improved the likelihood that an organ will be available. However, despite this improvement, the mortality rate for waitlist patients with acute end-stage exacerbations remains as high as 50% (4,5). The waitlist time for such individuals (median of 12 days) is heavily influenced by blood type, body size, and antibodies. An urgent exacerbation is most likely to develop in patients with idiopathic pulmonary fibrosis or cystic fibrosis, but it can also develop in patients with pulmonary hypertension, bronchiolitis obliterans syndrome, or chronic obstructive pulmonary disease.

In general, patients who present with acute exacerbations require aggressive noninvasive or invasive ventilation strategies. These patients either are already on the transplant list or require an emergent evaluation. Expeditiously deciding whether a patient is an appropriate lung transplant candidate is critical. If the patient is not improving or is worsening, then extracorporeal membrane oxygenation (ECMO) may be considered as a bridge to transplant or to decision. The decision to initiate ECMO should involve a multidisciplinary team to consider reasonable endpoints, cannulation strategies, management goals, and expected outcomes.

Indications

Any patient with refractory hypoxemia or hypercapnia despite optimal ventilatory support and adjunctive medical management is a potential candidate for ECMO (*Table 1*). Strategies often used to avoid ECMO include mechanical ventilation with 100% oxygen, positive end-expiratory

Table 1 Indications and contraindications for ECMO bridging

Indications
Refractory hypoxemia or hypercarbia or right heart failure despite optimal medical management in a patient who is a potential candidate for lung transplantation
Absolute contraindications
Ineligibility for lung transplantation according to standard criteria
Irreversible end-organ damage affecting multiple organs
Sepsis and bacteremia
Contraindications to systemic anticoagulation
Uncontrolled metastatic disease
Other terminal illness that is not otherwise treatable with a lung transplant
Acute intracerebral hemorrhage or stroke
Relative contraindications
Age >65 years
Limitations in vascular access
Obesity (BMI >30)
Frailty
Allosensitization with prolonged waitlist time
Prolonged ventilatory support

ECMO, extracorporeal membrane oxygenation; BMI, body mass index.

pressure, inhaled nitric oxide, inotropes, paralytics, steroids, and prone positioning. It is important to balance the risks of these interventions with the risks of ECMO. The need for ECMO should be anticipated so that it is placed electively rather than emergently, whenever possible.

The indication for ECMO is determined by the patient's candidacy for lung transplantation. If the patient is clearly not a candidate and has an irreversible process, then ECMO should be avoided. If the patient is already on the waitlist and irreversible end-organ damage or other conditions that would preclude him or her from remaining on the list have not developed, then ECMO is certainly indicated (6). The more challenging scenarios involve those patients who are somewhere in between, such as a patient who is not yet cleared for transplantation, or if it is unclear whether a patient's critical illness is reversible. In such cases, ECMO can be used as a bridge to decision. It is also important to consider the institution's resources and willingness to absorb the financial and regulatory risks involved in a potentially

adverse outcome. It can be helpful for institutions to partner with larger referral centers in their region that have accumulated experience with ECMO bridging.

Contraindications

Although contraindications may vary from program to program, several contraindications have been well established (*Table 1*). Absolute contraindications for ECMO bridging include the following: ineligibility for transplant according to standard criteria, irreversible end-organ damage affecting multiple organs, sepsis and bacteremia, contraindications to systemic anticoagulation, uncontrolled metastatic disease or another terminal illness that is not otherwise treatable with a lung transplant, and acute intracerebral hemorrhage or stroke.

Relative contraindications for ECMO bridging include the following: age greater than 65 years (because of impaired physiologic reserve), limitations in vascular access, obesity (body mass index >30), frailty, prolonged ventilatory support (i.e., >7 days), and allosensitization with prolonged anticipated waitlist time. Of note, prior lung transplantation is not, in and of itself, a contraindication for ECMO support (7).

In every case in which ECMO bridging is considered, each center will need to weigh the opinions of a multidisciplinary team consisting of a surgical ECMO specialist, lung transplant pulmonologist and surgeon, and critical care physician. Additional consultants should be included depending on the affected organ systems. Input from a physical therapist may also be helpful. Patients who have decompensated to the point that rehabilitation after ECMO is nearly impossible are unlikely to benefit from ECMO. Ambulatory ECMO is helpful for determining a patient's potential for rehabilitation after ECMO (8). Family member wishes and advanced directives are also critical to consider. End-organ dysfunction including renal, liver, or myocardial dysfunction is worrisome if it is unrelated to the patient's primary lung disease. For instance, hypoxemia and secondary pulmonary hypertension may improve with ECMO and transplantation, whereas fixed right ventricular dysfunction or fixed renal dysfunction will not. Patients who have been on the ventilator with aggressive support for greater than 7 days are also poor candidates for ECMO, underscoring the importance of anticipating ECMO support early. Resolving these issues in each instance can be difficult, and the consensus of the multidisciplinary group should be followed.
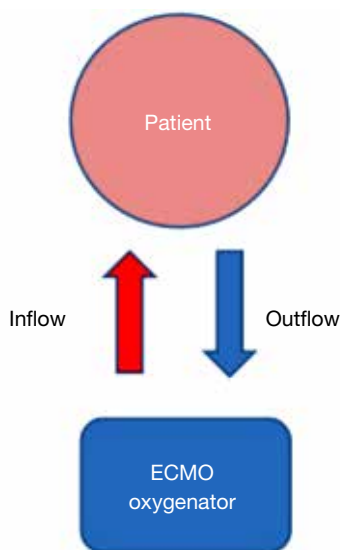
Figure 1 A common strategy for ECMO cannulation involving the use of femoral cannulation for the outflow (deoxygenated blood) and the femoral vein, internal jugular vein, or subclavian vein for the inflow (oxygenated blood) (Fem-IJ/SCV VV ECMO). ECMO, extracorporeal membrane oxygenation.

Technical considerations

Veno-venous (VV) and veno-arterial (VA) arrangements are used in ECMO support, both of which deliver blood from the patient to the ECMO oxygenator (outflow), and then from the oxygenator to the patient (inflow) (*Figure 1*).

In VV ECMO, the oxygenated blood goes to the right side of the heart and is pumped through the lungs to the left side of the heart, and finally out to the brain and body. In VA ECMO, oxygenated blood goes directly into the arterial circulation, thereby bypassing the pulmonary circulation.

VV ECMO support

VV ECMO is required for patients with severe lung disease who cannot oxygenate or remove CO_2 despite maximal ventilatory support. End-organ dysfunction may develop in these patients, as well as refractory acidosis or worsening pulmonary hypertension. Hemodynamic instability ensues, adding further insult to injury. Therefore, it is best to consider VV ECMO before these adverse events occur. A few fundamental requirements are as follows:

  (I)   Venous anatomy suitable for the cannulation strategy (venous Doppler is used to confirm that the right internal jugular vein or right subclavian vein is open);

  (II) Normal heart function (determined by using echocardiography);

  (III) No contraindication to anticoagulation;

  (IV) Reversible disease process;

  (V) Lack of significant resistance to pulmonary arterial flow (it can be less successful for patients with pulmonary hypertension or pulmonary fibrosis).

A common strategy is to use femoral cannulation for the outflow (deoxygenated blood), and the femoral vein, internal jugular vein, or subclavian vein for the inflow (oxygenated blood) (Fem-IJ/SCV VV ECMO) (*Figure 1*). For this procedure, the patient's neck and groin are fully prepped and draped. The veins are accessed with a large-bore introducer needle by using ultrasound guidance, and 100 to 200 units/kg of heparin are administered. A long J-wire is advanced through the femoral needle to the level of the right atrium, which is confirmed by using transesophageal echocardiography (TEE).

A series of dilators are passed over the wire before the femoral venous cannula (typically 22–26 F) is advanced to the level of the inferior vena cava (IVC). Importantly, the wire must not be looped; TEE can be used to help confirm this. The femoral cannula is much longer than the inflow cannula, which is typically a shorter arterial-type cannula (14–16 F). This cannula is advanced over a separate wire into the subclavian or internal jugular vein to the level of the superior vena cava (SVC). TEE is used to document these relative positions. The opposite femoral vein can be used if needed, but the risk of recirculation is greater.

The cannulas are clamped, carefully deaired, and connected to the ECMO circuit. Clamps are released, and ECMO is initiated. A chest radiograph is used to verify the location of the cannulas. Importantly, adequate separation between the two cannulas must be present; if not, recirculation can occur if the upper inflow cannula flows right into the lower outflow cannula. Oxygenation will be very poor in this situation, and the cannulas should be adjusted accordingly. If there is any concern for resistance across the pulmonary vasculature, a pulmonary vasodilator should be started. Another option is a right-sided Tandem with an oxygenator. The oxygenator on the ECMO circuit oxygenates the blood while the sweep feature removes CO_2. Typical PaO_2 levels just beyond the circuit are in the 400 to 450 mmHg range, whereas values in the periphery will range from 80 to 150 mmHg. Often, ventilator support is still required to maintain target oxygenation.

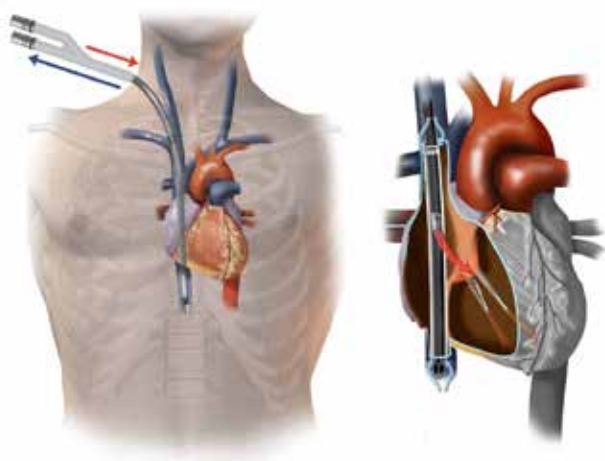The advantage of this cannulation strategy is that it can

Figure 2 Avalon cannula for veno-venous extracorporeal membrane oxygenation. The Avalon cannula removes blood through a proximal and distal port in the SVC and IVC. Oxygenated blood returns to the body through the middle inflow port oriented towards the tricuspid valve. Original figure by Hirose and colleagues (11), originally published by BioMed Central. Used with permission. SVC, superior vena cava; IVC, inferior vena cava.

be done at the bedside, if needed, or in the operating room. It is relatively straightforward for most cardiothoracic or general surgeons because they are familiar with the percutaneous wire technique. This is particularly useful when placing cannulas emergently at an outside facility (9). Also, the oxygenation tends to be excellent and highly predictable.

The downside of using this cannulation strategy is that femoral or IVC complications can be lethal. In an emergency, TEE may not be available, increasing the risk of vascular complications. Care must be taken to ensure the smooth passage of the femoral venous cannula because it can kink at the level of the subcutaneous tissue. The incisions are often small and can be closed with a deep single purse string suture (nonabsorbable) through the muscle, followed by an external pressure hold for 30 minutes. Alternatively, a femoral cutdown allows exposure to the vein if it is not identified percutaneously. Another important downside of using this approach is that the patient is immobile and cannot move with the groin and neck cannula in place.

The three-stage Avalon venous cannula (MAQUET Cardiovascular, LLC, Wayne, NJ) allows the patient to ambulate and is becoming popular for VV ECMO support (10) (*Figure 2*). The technique for insertion is similar to that described above, except that the right internal jugular vein is

most often used for access. The cannula can be large (27–31 F) and should be inserted with the use of fluoroscopy and TEE guidance. The internal jugular vein is accessed, and a wire is advanced into the infrahepatic IVC under fluoroscopy. If the wire does not traverse the IVC, a sheath is inserted, and a glide catheter and wire can be used to traverse the right atrial-IVC junction. The glide wire is then exchanged for a heavier wire (i.e., Amplatz or Lunderquist), and the Avalon venous cannula is advanced over the wire. The cannula is positioned as such that the inflow limb is towards the patient's neck, aligning the inflow port with the tricuspid valve (*Figure 2*) (11).

Flow through the tricuspid valve is confirmed by using TEE. To prevent recirculation, it is important to ensure that the SVC and IVC inflow ports are in their respective locations. The advantage of this approach is that the patient has the ability to mobilize. The disadvantage is that peripheral oxygenation is not always predictable and highly depends on the degree of pulmonary vascular resistance. Also, vascular complications such as IVC or right ventricular perforation can be catastrophic (11). It is best to have a practitioner with experience performing this technique under fluoroscopy.

VA ECMO support

Patients with indications for ECMO support who also have elevated pulmonary vascular resistance or cardiac dysfunction will require VA ECMO support. VA ECMO can be achieved through a variety of configurations. Essentially, an artery is used to deliver oxygenated blood (inflow) to the body, bypassing the pulmonary circulation, while a vein is used to deliver deoxygenated blood (outflow) to the ECMO oxygenator. Mobile ECMO can be achieved by placing a shorter percutaneous venous outflow cannula (22–24 F) in the internal jugular or subclavian vein to achieve the so-called "sports model". The arterial cannula is placed directly into the axillary artery (12), and the patient is prepped and draped. A 6-cm incision is made under the clavicle, and proximal and distal control of the axillary artery is achieved. After heparin is administered, the vessel is clamped, and an 8-mm polyester graft is sewn to the axillary artery and tunneled through the subcutaneous tissue to a small counter incision. The graft is connected to the ECMO tubing by using a 1/4 by 3/8-inch adapter and is secured with heavy ties and banding ties. The incision is closed with absorbable sutures. The key advantage to this technique is patient mobility. However, the main

disadvantage is the risk of limb hyperperfusion, which has been reported in up to 25% of cases but can be reduced by starting with lower ECMO flows (13).

In cases of severe primary or secondary pulmonary hypertension, other strategies are also available that take advantage of elevated right-sided pressures. The pumpless lung assist device involves a connection between the pulmonary artery and left atrium to bypass the lungs and provide oxygenation, decarboxylation, and right ventricular unloading (14,15). Also, a balloon septostomy has been used successfully in patients with VV ECMO support, allowing oxygenated blood to pass into the systemic circulation (16).

Central ECMO is achieved through a median sternotomy. A purse string is placed directly on the aorta with pledgeted sutures and also in the right atrium. After a small stab incision is made in the aorta, an arterial cannula (20–22 F) is inserted that is deaired and secured to the ECMO circuit. Likewise, a plastic venous cannula is inserted through the right atrium and secured. These cannulas are often externalized through counter incisions in the upper abdomen to allow for sternal closure. Central ECMO is used in cases in which the axillary artery is too small (i.e., <6–8 mm) or is insufficient to provide oxygenated blood to the periphery. The advantages of central ECMO are excellent oxygenation, low risk of stroke, and no risk of limb complication. The downsides are its invasiveness and lack of patient mobility, which can be addressed by externalizing the cannulas. A small right lateral thoracotomy can also be used to limit invasiveness, although it can be technically more challenging to perform.

Finally, femoral VA ECMO is a common arrangement for percutaneous placement, as described above for VV ECMO. The difference is that, for femoral VA ECMO, the inflow arterial cannula is inserted percutaneously or through an open cutdown into the common femoral artery. A cutdown is often required for closure of the arterial defect. The arterial cannula is typically 16 to 19 F, depending on the size of the femoral artery; the venous cannula is similar to that described above for femoral VV ECMO (12). The main advantage of femoral VA ECMO is the ease of placement. A disadvantage is that upper-body oxygenation will be compromised if the patient's cardiac function is good. The reason for this is that the poorly oxygenated blood from the left atrium and ventricle will be pumped out around the aortic arch. The femoral artery's contribution to oxygenation in this case will be limited to the lower half of the body, and a "watershed" will form at the level of the descending aorta— a condition often referred to as Harlequin syndrome (17).

This phenomenon is the reason why femoral VA ECMO is typically indicated only for patients in cardiogenic shock. One way to avoid this around this is to consider placing an additional inflow arterial cannula in the internal jugular or subclavian vein in a so-called "VVA" configuration (18). Other disadvantages of femoral VA ECMO include femoral artery complications such as bleeding or dissection, as well as patient immobility (17). Also, distal femoral perfusion cannulas are often used to prevent distal limb ischemia. If the femoral VA cannulas are in the same limb and a large venous cannula is used, venous congestion could lead to severe compartment syndrome.

In summary, VV ECMO is used for additional oxygenation and can be placed peripherally or through a 3-port system (Avalon) for mobility. VA ECMO is used in patients with elevated pulmonary vascular resistance, cardiac dysfunction, and pulmonary hypertension. The advantages and disadvantages of either method should be carefully considered, ideally through a team approach involving the surgeon, pulmonologist, and intensivist, as well as the patient and family. Moreover, other extracorporeal life support (ECLS) technologies are available for achieving specific goals in terms of decarboxylation, oxygenation, and hemodynamic support. For instance, CO_2 removal can be accomplished with the use of extracorporeal CO_2 removal (i.e., $ECCO_2R$). In addition, two types of Novalung (Novalung GmbH, Hechingen, Germany) configurations are available: the peripheral Novalung, which is an VA configuration that relies on the patient's cardiac output but allows decarboxylation and partial oxygenation; and the pulmonary artery to left atrium Novalung (i.e., PA-LA Novalung), which is a configuration that bypasses the lungs, relies on the patient's cardiac output, and provides decarboxylation and oxygenation. *Table 2* [adapted from (19) by Reeb and colleagues] summarizes these various ECLS technologies and their respective uses.

Management of ECMO

ECMO in patients waiting for a lung transplant is managed according to standard ECMO practices. Generally, in the rare event that recovery may occur, the lungs are allowed to rest with minimal tidal volume (typically <6 cc/kg) and positive end-expiratory pressure (5–10 mmHg). The lungs should be assessed periodically for recovery. ECMO support is weaned with optimal tidal volume ventilation while blood gasses and oxygen saturation are assessed. It is preferable—although not always feasible—for the patient to

Table 2 Summary of various ECLS technologies and their respective uses

ECLS technique	Oxygenation	Decarboxylation	Circulatory support
VV ECMO	Central organs	Yes	No
Peripheral VA ECMO	Peripheral organs	Yes	Yes
Central VA ECMO	Central organs	Yes	Yes
ECCO$_2$R	No	Yes	No
Peripheral Novalung*	No	Yes	No
PA-LA Novalung*	Yes	Yes	RV remodeling

*, Novalung GmbH, Hechingen, Germany. Adapted from the original and used with permission (19). ECLS, extracorporeal life support; VV, veno-venous; ECMO, extracorporeal membrane oxygenation; VA, veno-arterial; ECCO$_2$R, extracorporeal carbon dioxide removal; PA-LA, pulmonary artery to left atrium.

remain extubated. Noninvasive ventilation strategies may be used for this (5,20-23). If the patient is intubated, an early tracheostomy should be considered to allow participation in conditioning programs.

Lab values are checked every 4 h to ensure that coagulation is optimized. Standard hemoglobin thresholds are not uniformly agreed upon. In general, enough hemoglobin is needed to maintain good distal organ perfusion; we employ a threshold of 8 gm/dL before transfusion. The danger of overtransfusing a patient is that antibodies develop. The danger of undertransfusing is that end-organ dysfunction can be exacerbated.

Bleeding is a major complication with ECMO. Coagulation factors should be checked regularly and corrected as needed with additional products (21). Platelet levels should be kept at >50,000, the international normalized ratio at <1.8, and fibrinogen at >200 mg/dL. Heparin is used to maintain the activated clotting time between 160 and 200. Diuresis with medications and/or continuous renal replacement therapy is important to maintain a euvolemic status. ECMO is best managed by an intensive critical care team and an ECMO specialist, who is usually a perfusionist and/or a respiratory therapist trained in ECMO. This team, along with the bedside nurse, will make minute-by-minute adjustments on the basis of established center-specific protocols. The team works closely with pulmonologists and transplant surgeons to ensure that the global objective of maintaining end-organ oxygen delivery and possible pulmonary recovery is achieved.

Physical deconditioning is common with ECMO. To prevent this, physical therapy is critical. Paralytic agents and excessive steroids should be avoided. Every effort should be made to perform active range of motion exercises with the patient. Ambulatory ECMO is helpful for conditioning

and for assessing a patient's potential for recovery after transplantation (23). Ko and colleagues (24) showed that multiple physical therapy sessions including ambulatory ECMO are safe with the use of a mobilization screening protocol.

The goals of patient care should be reviewed regularly with the team and the patient's family. A palliative care consult is important early in the course of ECMO support. In general, if the patient is stable or improving, then ECMO can be reasonably continued. However, progressive worsening should prompt a discussion about the possible withdrawal of support. While there are no specific time limits on ECMO support, 14 days is typically the upper limit of support time before worsening end-organ status is observed. In the rare case of recovery, weaning trials can be used to assess the ability of the patient to separate from ECMO (20).

Outcomes

During the last decade, the outcomes of ECMO bridging have gradually improved. A report on the US trends in bridging outcomes by Hayanga and colleagues (25) showed that in 2000–2002, the 1-year survival rate after ECMO bridging was 25%, which was increased to 74% in 2009–2011. This may be because of improvements in circuit design, better management, or better patient selection (20). In their study, patients older than 35 years and those with cystic fibrosis or other diagnoses did worse. Patients who were bridged had a higher risk of dialysis-dependent renal failure. In every case, patients who were bridged did worse than those who were not bridged, but the gap in 1-year survival narrowed by the 2009–2011 era (74% *vs.* 86%). This difference in survival, however, must be considered in the context that patients who are not bridged have a 100%

mortality rate without a transplant.

Because old age increases the risk of perioperative mortality, older patients should be approached with caution. The report by Hayanga and colleagues (25) showed that age greater than 35 years was an independent risk factor in patients who were bridged. Nonetheless, a follow-up case report and literature review described a successful transplantation for a 70-year old patient who was bridged with conscious sedation and no mechanical ventilation (26). This highlights the importance of patient selection, the optimization of the bridging strategy, and the careful weighing of competing risk factors, rather than having a strict cutoff. In general, any patient older than 65 years should have very few or no additional risk factors to be considered for bridging to transplantation.

Transplant volume may also be an important factor in determining outcomes for bridging. In a United Network for Organ Sharing (UNOS) review, Hayanga and colleagues (27) showed an adjusted hazard ratio for mortality of 2.74 for patients who were bridged to lung transplantation with ECMO in a low-volume center (i.e., 1–5 transplants/year) versus a high-volume center (i.e., >15 transplants/year). This is an important consideration and suggests that transportation from a low-volume center to a higher-volume center may be wise, from both a risk and quality standpoint.

In 2012, a study by Lang and colleagues (28) in Vienna, Austria showed a 90% success rate for patients who were bridged to transplantation but a 24% rate of in-hospital mortality after transplantation; median bridging time was 5.5 days (range, 1–63 days). Patients who were bridged and survived the initial 3-month period after transplantation had a 5-year survival rate that was equivalent to that of patients who were not bridged (63% *vs.* 72%, P=0.33). This again emphasizes the importance of selecting patients who are most likely to tolerate the perioperative insult of ECMO. It also underscores the significance of optimizing patients on ECMO and knowing when the patient is making a turn for the worse and may no longer be a good candidate.

In a review of 26 cases of bridging to transplantation, Weig and colleagues (29) showed a success rate that was lower than that reported by the Vienna group (50% *vs.* 90%). Median time on ECMO was 33 days (range, 17–55 days). No notable differences were observed between patients who survived to transplantation and those who did not. In addition, Weig and colleagues (29) studied several potential risk factors and found that patients who did not survive lung transplantation after bridging had higher bilirubin levels, pulmonary artery pressures, and sequential organ failure

assessment (SOFA) scores than did the surviving patients. A bilirubin level >3 mg/dL and a SOFA score >9 predicted a uniformly fatal outcome. Again, these are high-risk features that need to be carefully considered before committing to transplantation and when considering ECMO as a bridge to transplantation.

A study by Crotti and colleagues (30) showed a successful bridging rate of 68%. Time on ECMO was an independent factor in predicting survival after transplantation, with patients who underwent transplantation after less than 14 days of ECMO having a 100% 1-year survival rate and patients who were on ECMO for more than 14 days before transplantation having a 50% 1-year survival rate. Mean SOFA scores from the initiation of ECMO to the end of ECMO went from 5.6 to 6.7 in the early group and from 5.2 to 9.7 in the late group. The patients on noninvasive ventilation before transplantation had a 20% mortality rate while on the waitlist and a 60% 1-year survival rate after transplantation, whereas patients requiring intubation before transplantation had a 40% mortality rate while on the waitlist and a 47% 1-year survival rate after transplantation.

Mason and colleagues (31) showed a successful bridging rate of 74%. They observed that patients who were bridged had a significantly longer hospital stay, greater coagulopathy, and higher rates of dialysis and tracheostomy. Despite this, they saw no difference in 3-year survival rates between patients who were bridged with ECMO and those who were not. Several important complications resulted in death while patients awaited transplantation on ECMO, including renal failure (21%), sepsis (16%), diffuse intravascular coagulopathy (10%), anoxic brain injury (5%), and multisystem organ failure (5%). Patient morbidities after transplantation were also significant and included open chest management (50%), continuation of ECMO (21%), and reoperation for bleeding (29%).

In 2013, 11 centers in France combined data from 36 patients who were bridged with ECMO into a registry report. Their cumulative success with bridging was 83%; however, only 56% of patients were discharged from the hospital (32). Furthermore, only 47% of patients who were bridged were living at 17-month follow-up. Cystic fibrosis patients had the best survival, with a 56% survival rate at 3 years from the initiation of ECMO. This was contrary to the report by Hayanga and colleagues (25). Toyoda and colleagues (33) reported a 77% success rate in 31 patients who were bridged with ECMO. The median duration of ECMO was 91 h. They noted significantly higher rates of PGD3 requiring ECMO support (54% *vs.* 6%) and a longer

Table 3 Factors that affect post-transplant survival in patients on ECMO support

Favorable factors
Age <50 years
Normal or marginally elevated total bilirubin
Normal or mildly elevated pulmonary artery pressures
<14-day duration on ECMO
Low SOFA score (<6)
Non-invasive ventilation
Ability to participate in physical therapy (i.e., "awake ECMO")
Unfavorable factors
Age >60 years
Total bilirubin >3
Severe pulmonary hypertension
Prolonged ECMO (>14 days)
Prolonged mechanical ventilation
Prolonged immobility on ECMO
SOFA score >9
Major bleeding, infectious complications, or end-organ complications on ECMO
Retransplant with a retransplant interval <1 year

SOFA, sequential organ failure assessment; ECMO, extracorporeal membrane oxygenation.

median hospital stay (46 *vs.* 27 days) in the bridged group than in the non-bridged group. Despite this, no significant difference was observed in the 2-year survival rate after transplantation, regardless of preoperative ECMO status (74% for both preoperative ECMO and no ECMO).

Collaud and colleagues (7) performed a literature review and pooled analyses to assess the role of ECMO bridging in retransplantation. They found that the 1-year overall survival rate was 48%. The intertransplant interval was a significant factor affecting survival in these patients. For the subgroup of patients with an intertransplant interval of >2 years and who were bridged on awake ECMO (ambulatory, communicating, low-vent requirements), the 1-year survival rate was 67%.

Another study performed at Zurich University Hospital in Switzerland, showed an 86% successful bridging rate. Intensive care unit and ventilation times were significantly longer in patients bridged to transplantation than in controls who were not bridged (34). The rates of PGD3 and

mortality at 2 years were also higher for bridged patients. For a subgroup of patients bridged on awake ECMO, all of them were living at a median follow-up time of 10.8 months. Similarly, Lang and colleagues (28) showed that patients bridged with awake ECMO had a 2-year survival rate of 60%, compared with a 2-year survival rate of 29% for patients bridged with ventilation, sedation +/− ECMO. Thus, evidence indicates that awake ECMO is a good prognostic indicator for patients who can tolerate it.

Biscotti and colleagues (4) reported their 9-year experience at Columbia Presbyterian and described a 55% success rate when bridging with ECMO. They identified several factors by using univariate analysis that predicted the likelihood of whether a patient will survive to transplantation. A higher percentage of inotrope or vasopressor use was noted in the non-survival group. In addition, a higher simplified acute physiology II score and a lower rate of ambulation were found in the group that was not successfully bridged. Consistent with the French experience described above, patients with cystic fibrosis had the most favorable prognosis for surviving to transplantation. Also, the need for renal replacement therapy was higher in the group of patients who did not receive a transplant. Cystic fibrosis patients had the best rate of survival after transplantation, whereas patients with interstitial lung disease had the worst rate of survival after transplantation.

Table 3 summarizes the favorable and unfavorable traits of patients on ECMO that can be considered to help predict whether an outcome will be successful after transplantation. This is based on the consolidation of the above-referenced data, as well as on our own institutional experience, but it should not be used in isolation to decide who should undergo transplantation, given that the literature in the field is still evolving.

Conclusions

There are two fundamental questions that one faces when deciding which patients to place on ECMO: (I) is this patient a good candidate for ECMO? (II) Should this patient be transplanted off of ECMO? For the first question, any patient who is even remotely close to being considered a transplant candidate and who has refractory hypoxemia or hypercapnia should be offered ECMO support. At the minimum, this allows a bridge to decision. For the second question, several considerations have been described in the outcomes section above and in *Table 3* that help guide daily

multidisciplinary discussions and decisions. There is not a commitment to transplantation just because the patient is on ECMO. Discussions among a multidisciplinary team and the patient's family should occur daily to weigh the patient's quality of life and the chance of survival.

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Footnote

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References

1. Blivet S, Philit F, Sab JM, et al. Outcome of patients with idiopathic pulmonary fibrosis admitted to the ICU for respiratory failure. Chest 2001;120:209-12.

2. Mollica C, Paone G, Conti V, et al. Mechanical ventilation in patients with end-stage idiopathic pulmonary fibrosis. Respiration 2010;79:209-15.

3. Rangappa P, Moran JL. Outcomes of patients admitted to the intensive care unit with idiopathic pulmonary fibrosis. Crit Care Resusc 2009;11:102-9.

4. Biscotti M, Gannon WD, Agerstrand C, et al. Awake Extracorporeal Membrane Oxygenation as Bridge to Lung Transplantation: A 9-Year Experience. Ann Thorac Surg 2017.

5. Gottlieb J, Warnecke G, Hadem J, et al. Outcome of critically ill lung transplant candidates on invasive respiratory support. Intensive Care Med 2012;38:968-75.

6. Rajagopal K, Hoeper MM. State of the Art: Bridging to lung transplantation using artificial organ support technologies. J Heart Lung Transplant 2016;35:1385-98.

7. Collaud S, Benden C, Ganter C, et al. Extracorporeal Life Support as Bridge to Lung Retransplantation: A Multicenter Pooled Data Analysis. Ann Thorac Surg 2016;102:1680-6.

8. Biscotti M, Sonett J, Bacchetta M. ECMO as bridge to lung transplant. Thorac Surg Clin 2015;25:17-25.

9. Lee SG, Son BS, Kang PJ, et al. The feasibility of extracorporeal membrane oxygenation support for inter-hospital transport and as a bridge to lung transplantation. Ann Thorac Cardiovasc Surg 2014;20:26-31.

10. Garcia JP, Iacono A, Kon ZN, et al. Ambulatory extracorporeal membrane oxygenation: a new approach for bridge-to-lung transplantation. J Thorac Cardiovasc Surg 2010;139:e137-9.

11. Hirose H, Yamane K, Marhefka G, et al. Right ventricular rupture and tamponade caused by malposition of the Avalon cannula for venovenous extracorporeal membrane oxygenation. J Cardiothorac Surg 2012;7:36.

12. Biscotti M, Bacchetta M. The "sport model": extracorporeal membrane oxygenation using the subclavian artery. Ann Thorac Surg 2014;98:1487-9.

13. Chamogeorgakis T, Lima B, Shafii AE, et al. Outcomes of axillary artery side graft cannulation for extracorporeal membrane oxygenation. J Thorac Cardiovasc Surg 2013;145:1088-92.

14. Strueber M, Hoeper MM, Fischer S, et al. Bridge to thoracic organ transplantation in patients with pulmonary arterial hypertension using a pumpless lung assist device. Am J Transplant 2009;9:853-7.

15. Schmid C, Philipp A, Hilker M, et al. Bridge to lung transplantation through a pulmonary artery to left atrial oxygenator circuit. Ann Thorac Surg 2008;85:1202-5.

16. Kon ZN, Pasrija C, Shah A, et al. Venovenous Extracorporeal Membrane Oxygenation With Atrial Septostomy as a Bridge to Lung Transplantation. Ann Thorac Surg 2016;101:1166-9.

17. Rupprecht L, Lunz D, Philipp A, et al. Pitfalls in percutaneous ECMO cannulation. Heart Lung Vessel 2015;7:320-6.

18. Ius F, Sommer W, Tudorache I, et al. Veno-veno-arterial extracorporeal membrane oxygenation for respiratory failure with severe haemodynamic impairment: technique and early outcomes. Interact Cardiovasc Thorac Surg 2015;20:761-7.

19. Reeb J, Olland A, Renaud S, et al. Vascular access for extracorporeal life support: tips and tricks. J Thorac Dis 2016;8:S353-63.

20. Combes A, Bacchetta M, Brodie D, et al. Extracorporeal membrane oxygenation for respiratory failure in adults. Curr Opin Crit Care 2012;18:99-104.

21. Garcia JP, Kon ZN, Evans C, et al. Ambulatory veno-venous extracorporeal membrane oxygenation: innovation and pitfalls. J Thorac Cardiovasc Surg 2011;142:755-61.

22. Gattinoni L, Carlesso E, Langer T. Clinical review: Extracorporeal membrane oxygenation. Crit Care 2011;15:243.

23. Mangi AA, Mason DP, Yun JJ, et al. Bridge to lung transplantation using short-term ambulatory extracorporeal membrane oxygenation. J Thorac

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Loor et al. Bridging to lung transplantation with ECMO

Cardiovasc Surg 2010;140:713-5.

24. Ko Y, Cho YH, Park YH, et al. Feasibility and Safety of Early Physical Therapy and Active Mobilization for Patients on Extracorporeal Membrane Oxygenation. ASAIO J 2015;61:564-8.

25. Hayanga AJ, Aboagye J, Esper S, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation in the United States: an evolving strategy in the management of rapidly advancing pulmonary disease. J Thorac Cardiovasc Surg 2015;149:291-6.

26. Hayanga JA, Murphy E, Girgis RE, et al. Extracorporeal Membrane Oxygenation as a Bridge to Lung Transplantation in Patients Over Age 70 Years: A Case Report. Transplant Proc 2017;49:218-20.

27. Hayanga JW, Lira A, Aboagye JK, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation: what lessons might we learn from volume and expertise? Interact Cardiovasc Thorac Surg 2016;22:406-10.

28. Lang G, Taghavi S, Aigner C, et al. Primary lung transplantation after bridge with extracorporeal membrane oxygenation: a plea for a shift in our paradigms for indications. Transplantation 2012;93:729-36.

29. Weig T, Irlbeck M, Frey L, et al. Parameters associated with short- and midterm survival in bridging to lung transplantation with extracorporeal membrane oxygenation. Clin Transplant 2013;27:E563-70.

30. Crotti S, Iotti GA, Lissoni A, et al. Organ allocation waiting time during extracorporeal bridge to lung transplant affects outcomes. Chest 2013;144:1018-25.

31. Shafii AE, Mason DP, Brown CR, et al. Growing experience with extracorporeal membrane oxygenation as a bridge to lung transplantation. ASAIO J 2012;58:526-9.

32. Lafarge M, Mordant P, Thabut G, et al. Experience of extracorporeal membrane oxygenation as a bridge to lung transplantation in France. J Heart Lung Transplant 2013;32:905-13.

33. Toyoda Y, Bhama JK, Shigemura N, et al. Efficacy of extracorporeal membrane oxygenation as a bridge to lung transplantation. J Thorac Cardiovasc Surg 2013;145:1065-70; discussion 70-1.

34. Inci I, Klinzing S, Schneiter D, et al. Outcome of Extracorporeal Membrane Oxygenation as a Bridge To Lung Transplantation: An Institutional Experience and Literature Review. Transplantation 2015;99:1667-71.

Bilateral versus single lung transplantation: are two lungs better than one?

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Abstract: There is a long-standing debate over whether single or bilateral lung transplant provides better short and long-term clinical outcomes. We performed a detailed PubMed search on relevant clinical research publications on single (SLT) and bilateral lung transplantation (BLT). We included studies that were published before and after the implementation of the lung allocation score (LAS). We reviewed disease-specific short- and long-term outcomes associated with each transplantation technique. The majority of published studies are retrospective cohort studies that use institutional data or large patient registries. Outcomes associated with transplantation technique vary by disease specific indication, age, and patient severity. Over the past decade, the relative proportion of bilateral lung transplantation has increased. Increasing adoption of bilateral lung transplant likely reflects the general acceptance of several advantages associated with the technique. However, making a clear, evidence-based decision is difficult in light of the fact that there has never been and probably never will be a randomized trial. Our institutional preference is bilateral lung transplant. However, consideration for the technique should still be made on a case-by-case basis.

Keywords: Bilateral; single; lung transplantation; outcomes

Introduction

Since the first successful isolated lung transplant performed by Dr. Joel Cooper at the University of Toronto in 1983, lung transplantation has been considered an optimal therapy for multiple causes of end stage pulmonary disease (1). The initial isolated transplant operations were single lung transplants performed on patients with severe idiopathic pulmonary fibrosis (IPF). Since then, lung transplantation has been more heavily utilized to treat patients with multiple conditions including interstitial lung disease (ILD), chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pulmonary hypertension, and more (2). Over the past several decades, changes in donor selection, postoperative care, and immunosuppression therapy have broadened the use of lung transplant and improved

outcomes for transplant recipients (3). While new guidelines have been created to help guide transplant candidate selection and management, there is still substantial debate surrounding the utilization of single versus bilateral lung transplantation in patients eligible for either strategy (2,4,5). To date, much of the decision-making regarding use of single versus bilateral lung transplant is based on individual institutional case series experience or retrospective reviews of large lung transplant registries. There is a lack of high quality, prospective data to provide clear criteria favoring single or bilateral lung transplantation when either strategy is possible. Furthermore, there is a lack of consensus on the philosophical dilemma: should a bilateral operation with better palliation be offered to fewer patients, or should a lesser unilateral operation be offered to more recipients? The purpose of this chapter is to review the existing

literature regarding single and bilateral lung transplantation. Specifically, this review will highlight the following subjects:

❖ Disease-specific indications for single (SLT) *vs.* bilateral lung transplantation (BLT), with a focus on emphysema and pulmonary fibrosis;
❖ Impact of procedure type on post-transplantation functional status;
❖ Impact of procedure type on post-transplantation quality of life (QOL);
❖ Chronic rejection after lung transplantation;
❖ Ethical challenges facing the choice between single and bilateral transplants;
❖ The novel strategy of "staged BLT (SBLT)".

Disease-specific indications for lung transplantation

There is a wide variety of indications for lung transplantation, including end-stage COPD, ILD, pulmonary hypertension, CF and bronchiectasis and others (2). Because patients with septic lung disease (including CF and bronchiectasis) almost always undergo BLT due to the infectious risk posed by the retained native lung, they will not be discussed further in this chapter (6). The International Society for Heart and Lung Transplantation (ISHLT) provides the most comprehensive data on long-term survival associated with BLT and SLT for all recipients (7). They collect data from 256 lung transplant and 180 heart-lung transplant centers, and represent an estimated 75% of international thoracic transplant activity. The registry is ideal for examining longitudinal trends, as the registry requires submission of follow-up data on a yearly basis. In the 2017 ISHLT report summarizing survival trends from 1990–2015, recipients of a lung transplantation operation had a median survival of 6.0 years. In unadjusted analysis, BLT recipients had better survival post-transplant compared to SLT recipients. This difference was first seen at 1 year post-op, but increased over a 14-year follow-up period. Survival for BLT and SLT groups were 90% and 88% at 3 months, 82% and 78% at 1 year, 69% and 61% at 3 years, 59% and 48% at 5 years, and 41% and 23% at 10 years, respectively. That high level comparison simply begins the discussion, but there are multiple issues of selection bias and confounding that cloud the comparison of single or bilateral transplantation outcomes. Additional literature has focused on short and long-term outcomes associated with transplantation type within subgroups of patients with specific diagnoses. Much of the existing literature examines the use of SLT and BLT in patients with either advanced COPD or IPF.

COPD

Emphysema (which encompasses COPD and alpha-1 antitrypsin deficiency) has been the most common indication for lung transplantation (2). The first successful experience with transplantation in the COPD population involved isolated SLT, initially described by Dr. Joel Cooper and his team (1). However, with the development of BLT and improvements in technique, BLT has received increased clinical adoption and use in patients with COPD (6,8). The prevailing physiologic reasoning supporting use of BLT is that the technique reduces the risk of early ventilation/perfusion mismatch and eliminates the issue of subsequent hyperinflation in the unresected emphysematous native lung that occurs after SLT (8). During the accumulation of the early experience, there was a tendency to offer BLT to younger patients with the notion that they might have greater physiologic reserve to be able to withstand the increased stress of a more prolonged surgery (1,4,6). That selection bias might have also burdened the SLT cohort with an older and frailer group of patients who would be at greater risk for premature death regardless of the differential contribution of SLT versus BLT.

Meyer and colleagues in 2001 performed one of the early index studies comparing BLT and SLT in the COPD population (4). Using the ISHLT/United Network for Organ Sharing (UNOS) registry, they performed a retrospective analysis of patients with COPD undergoing lung transplantation. They attempted to study the correlation between transplantation technique (SLT *vs.* BLT) and survival, stratified by age (41–50, 51–60, 61–70 years). They identified 2,260 lung transplant recipients (1,835 SLT, 425 SBLT) from 1991–1997 and performed risk-adjusted survival analysis using Cox regression, Kaplan-Meier analysis, and calculation of risk ratios for mortality. Among all transplant recipients, recipient age and procedure type (SLT *vs.* BLT) were found to be associated with increased risk for mortality, with advanced age, SLT, and their interaction demonstrating significant associations. On Kaplan-Meier analysis, the authors demonstrated that BLT was associated with higher survival in both the 41–50 and 51–60 years age categories across all time points, with a more pronounced survival benefit occurring further out from surgery. Survival rates among younger patients (<50 years) who underwent SLT

were 93.6%, 80.2% and 43.6% at 30 days, 1 year, and 5 years, respectively, compared to 94.9%, 84.7%, and 68.2% in the young BLT group (P=0.001). Among those aged 51–60 years, the differences in long-term survival were slightly less pronounced. Those who received SLT had 30-day, 1 year, and 5-year survival rates of 93.5%, 79.4%, and 39.8%, respectively, compared to 93.0%, 79.7%, and 60.5% for patients of similar age who received BLT (P=0.05). After age 60, however, the trend reversed. Survival associated with SLT was considerably higher (93.0%, 72.9%, and 36.4%) compared to BLT (77.8%, 66.0%, with 5-year mortality data unavailable) (P=0.2). When using risk ratios to calculate risk of mortality across all ages, the authors noted an increased probability of mortality for recipients of SLT between ages 40–57 (P=0.001 at each age). At approximately age 57, the trend reversed. Additionally, the authors focused on US transplant cases to examine 3-year morbidity associated with transplantation technique. They measured events of hospitalization for rejection, onset of bronchiolitis obliterans (BOS), bronchial airway complications, and hospitalization for infections. No significant differences were observed between BLT and SLT in any of these measures. However, the study did not measure variables associated with short-term morbidity, which may be more relevant in older patients. The study concluded that BLT was associated with greater short and long-term survival in patients less than 60 years of age.

Thabut and colleagues confirmed the positive long-term survival advantage that BLT offered to the younger COPD population (9). They completed a large retrospective analysis of the ISHLT registry between 1987 and 2006. Thabut performed a survival analysis of 9,883 patients with a diagnosis of COPD. Additionally, they documented important trends in the use of BLT for COPD. For example, the proportion of patients with COPD who underwent BLT more than doubled from the 1990s to more recently (21.6% in 1993 to 56.2% in 2006). Using modern propensity score matching (a technique lacking in previous papers on the subject) to control for possible treatment selection bias associated with each transplant method, Thabut determined that median survival time was significantly greater for those who received BLT (6.41 years, 6.02–6.88 years) compared to SLT (4.59 years, 4.41–4.76 years) (P<0.0001). However, the survival advantage associated with BLT did not hold for patients greater than 60 years of age. The practical suggestion was similar: to offer BLT to younger COPD patients but to accept the lack of a difference in older

recipients and perhaps use other criteria to choose the transplantation strategy in this population.

The authors' institution (Washington University in St. Louis/Barnes Jewish Hospital) is a high-volume lung transplantation center, and BLT has been the preferred transplantation method. Cassivi performed a 13-year review of lung transplantation for COPD patients at Barnes-Jewish Hospital between 1988 and 2000 looking at in-hospital mortality and 5-year survival rates among patients with COPD and alpha-1-antitrypsin deficiency (10). More than 70% of emphysema patients received BLT, reflecting the strong institutional preference for the method. Cassivi acknowledged that the preference for BLT was due to a record of increased survival and ease of postoperative ventilator management. When examining long-term survival, COPD patients who received BLT had significantly higher 5-year survival at 66.7% compared to 44.9% for single lung replacement (P<0.001). Conversely, many other studies did not find the same survival benefit conferred by BLT.

Bennett and colleagues performed a retrospective single center review of COPD patients undergoing lung transplantation, with a special focus on patients older than 55 years of age (11). These authors noted that it was standard policy since the inception of their transplantation program to only perform SLT on emphysema patients older than 55 years of age. They attempted to identify specific patient subgroups that benefit from SLT. They examined 5-year survival rates between patients receiving SLT (206 patients) and BLT (30 patients) from 1992–2012. As expected, the SLT cohort tended to be older and had reduced pre-transplant pulmonary function and physical conditioning compared to the BLT cohort. Within this institution, 30-day, 1-year, and 5-year survival estimates between treatment cohorts were similar, with long-term survival trending slightly higher for BLT patients. Due to their small pool of BLT patients, they also compared their institutional data to the outcomes of SLT and BLT patients in the UNOS registry. When comparing institutional data to SLT and BLT patients in the UNOS registry, Bennett noted that their own institution's SLT patients had generally similar preoperative risk in terms of advanced age, comorbid condition, and pulmonary function. Their institution's SLT short and long-term survival rates were similar to those of the UNOS registry's BLT subset. This may reflect improved experience and perioperative care, given that SLT is their institutional preference. They concluded that while BLT may provide an individual survival benefit, SLT

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had substantial utility and should be promoted as much as possible given the overall impact that it can have in increasing the number of patients receiving transplantation.

Several studies of BLT *vs.* SLT were performed on institutional or national databases that captured data before the 2005 implementation of the lung allocation score (LAS). Schaffer compared SLT and BLT in the post-LAS era (12). Using the UNOS registry from 2005-2012, Schaffer compared graft survival between transplantation types. Graft survival represented a composite of post-transplantation mortality and graft failure rates. These patients were propensity matched to reduce the impact of treatment selection bias on the results of the study. Among 3,174 COPD patients, 1,299 underwent SLT and 1,875 underwent BLT. The median follow-up was carried over 2 years post-transplant, and there was no significant association found between type of transplant and median graft survival (67.7 months for BLT *vs.* 64.0 months for SLT; P=0.23). This distinction from previous study results may be explained by the novel way that patients were selected for transplantation using the LAS. Compared to the pre-LAS era, during which time on the waiting list gave priority for transplantation, patients with COPD in the LAS era must be comparatively more impaired to achieve a higher transplantable score (13). The use of the LAS to prioritize recipients for transplantation may have reduced the apparent benefit of BLT for patients with COPD.

ILD and interstitial pulmonary fibrosis (IPF)

ILD, which includes IPF, carries the worst overall prognosis among end stage pulmonary disease indications for lung transplantation (14). Median survival time for patients with IPF ranges from 2–3 years post diagnosis without lung transplantation, with 5-year post-transplant survival rates ranging from 30–50% (14,15). Non-surgical therapies are limited (14,16). Lung transplantation has thus far been the only restorative therapy to offer a proven survival benefit. The short natural history of IPF without transplantation gave that diagnosis a competitive edge when the LAS was rolled out in 2005. With the application of the LAS in the United States, the rate of lung transplantation in this IPF population has risen dramatically. Despite the rising number of lung transplants in patients with IPF, there is no definitive survival advantage consistently shown to be associated with either BLT or SLT. Overall, however, the use of BLT in patients with IPF is on the rise. In 2011, approximately 54% of lung transplant operations among IPF patients were

bilateral (17). In a retrospective institutional case series performed at Cleveland Clinic, Mason and colleagues [2007] studied 82 patients who underwent lung transplantation for IPF (18). They compared overall 30-day, 1-year, and 5-year survival between patients with IPF and propensity-matched, non-IPF patients. Overall survival among IPF patients was significantly worse at all time points compared to their non-IPF matched counterparts. Additionally, they calculated that BLT conferred a survival advantage among IPF patients (81% *vs.* 67% and 55% *vs.* 34% at 1 and 5 years, respectively). However, they could only compare BLT versus SLT in 10 matched pairs due to the strong selection bias attributed to their institutional preference to perform BLT in younger patients. Interestingly, they failed to note advanced age as an independent risk factor for mortality in BLT.

Additional studies have also supported the use of BLT in IPF patients because of an apparent survival advantage. Weiss focused on transplantation in IPF patients after the institution of the LAS score (19). They examined all-cause mortality 1-year after transplant in 1,256 IPF patients listed in the UNOS registry between 2005 and 2007. Additionally, they further examined the effect of pre-transplant disease severity on mortality outcomes by stratifying patients into LAS quartiles. Quartiles 1–3 indicated lower risk IPF patients, while quartile 4 contained the highest risk IPF patients. They determined that IPF patients with higher LAS were more likely to receive BLT. They observed a trend towards greater usage of BLT in sicker patients, with 21% more patients receiving BLT in the highest LAS quartile compared to the lowest (59.5% *vs.* 38.4%, P<0.05). Within the highest quartile, SLT was associated with a 14.4% increased risk in cumulative mortality compared to BLT. However, in the lowest quartile, SLT was found to be an independent protective factor in terms of mortality. There was no demonstrated short-term survival benefit associated with either transplantation type. Their findings are counterintuitive to the notion that BLT should be reserved for younger patients with more physiologic reserve, and instead suggest a role for BLT specifically for those with potentially higher pre-operative risk.

Force conducted one of the largest retrospective reviews of lung transplantation among IPF patients (20). This report also demonstrated a survival advantage associated with BLT among IPF patients. The authors performed a retrospective review of the UNOS registry from 1987 to 2008 studying 3,860 patients (2,431 SLT and 1,429 BLT) using propensity score matching. Propensity-matched analysis failed to show

a substantial survival benefit for BLT (HR 0.90, 95% CI, 0.78–1.0, P=0.11). However, when a one-year conditional survival analysis was performed, the authors found that BLT had significantly better long-term survival (12.08 versus 6.8 years, P=0.0006). When analyzing for risk factors for death within the BLT group, they reported recipient age, donor age, and year of transplantation to be significant predictors of mortality. Specifically, they observed that patients over the age of 57 had higher 1-year post-transplant mortality risk. Based on the conditional survival analysis and the significant correlation between advanced age and mortality risk, the authors concluded that younger IPF patients would most likely benefit from BLT to enhance long-term survival.

Not all available studies found a survival advantage associated with BLT. Chauhan performed a review of the UNOS registry from 2001–2009, examining actuarial post-transplant graft survival (21). In a unique approach, they studied 1,001 lung transplant recipients with IPF who were concurrently listed for BLT and SLT. Four hundred thirty-four (43%) of these patients underwent SLT while the remaining 57% underwent BLT. The authors noted significant differences in baseline comorbidities, functional status, pulmonary function tests, and recipient disease severity. Despite these baseline differences, there were no observed differences in short or long-term graft survival. Based on these comparable outcomes, the authors advocated for more liberal use of SLT among IPF patients. However, they did note that a major limitation of their study was the assumption that organ assignment was random and based solely on the availability of one or two donor lungs. At the institutional level, or even the surgeon level, there may be great variability in willingness to accept any individual donor lung based on several donor and recipient characteristics. For example, a hospital may list a patient for either SLT or BLT, suggesting equipoise, but that same group may have a low threshold to decline a single lung donor. This effectively would make their original assumption about the equivalence of the transplanted lungs less valid.

Meyer performed a large-scale retrospective review of the early UNOS registry experience in 2001 that included a cohort of 821 lung transplant patients (636 SLT, 185 BLT) with pulmonary fibrosis (4). They produced an age-stratified comparison of survival by procedure type. On crude univariate analysis, they found that younger IPF patients (30–49 years) with SLT had better short and long-term survival post-transplant than similar patients after

BLT (90.9% *vs.* 77.1% at 1 month; 63.8% *vs.* 46.2% at 3 years; P=0.02). The same trend favoring single lung replacement was observed in older patients. However, when a 1-month post-transplant conditional survival analysis was performed, there were no significant subsequent differences seen between procedure types at any age group. This suggests that there may be greater periprocedural mortality associated with BLT. Propensity score matching and multivariate regression analysis failed to show survival differences between procedure types. Nwakanma focused their analysis on bilateral versus single lung transplants in IPF patients older than 60 years of age (22). Performing a large-scale analysis of 1,656 IPF patients in the UNOS registry between 1998 and 2004, they concluded that SLT was favored in this age group, with 78% of the patients in that sample undergoing SLT. Propensity score analysis demonstrated similar short and median-term survival between BLT and SLT. Transplantation type was not associated with mortality. Thus, they could not advocate for the use of either procedure type in older IPF patients.

When examining diagnosis-specific survival outcomes for BLT versus SLT, the existing literature demonstrates mixed findings. Comparing bilateral and single lung transplant effects by indication is crucial as the underlying pathophysiology of each disease is very different, and could greatly affect outcomes. The use of bilateral transplant for both COPD and IPF is on the rise. Both techniques have been utilized in younger and older populations despite previous notions that older individuals may "lack the reserve" to tolerate the procedure (4,9,10,19,20). Some data have demonstrated a greater advantage for using bilateral transplant in younger COPD populations, but the evidence in that disease is still conflicting (4,9). The picture is even more mixed in analyses of IPF patients. The available literature is relatively lackluster because most studies are small, retrospective, single-center case reviews. These studies are often limited in sample size and may be affected by institutional comfort and experience with a preferred technique. Other studies have relied on large retrospective database analysis of the ISHLT and UNOS registries, and many are based on data obtained before the institution of the LAS prioritization scheme. With the implementation of the LAS, the patient characteristics of those undergoing transplant are different, with a priority given to those with higher severity of illness instead of longer time spent on the waitlist (12). A randomized control trial is neither practical nor feasible in this setting. High quality, prospectively collected data collected from a variety of institutions that

comprehensively take into account the effects of age and multiple comorbidities will be useful in further unmasking the effect of transplantation type for advanced COPD and IPF patients. Until that time, the data are diverse and conflicting enough to simply state that there is equipoise between the two strategies. Factors other than patient survival or graft survival must be considered as well.

Post-transplant functional status and procedure type

In addition to collecting data on short- and long-term survival, several authors have examined the influence of BLT versus SLT on post-transplantation functional status. Functional status is most commonly quantified by spirometry, which has been strongly correlated with QOL in lung transplant patients (23). However, other measures such as the 6-minute walk test (6MWT) and comprehensive surveys on each patient's ability to perform daily activities have also been used. Mason and colleagues performed a single institution study of the relative impact of lung transplantation on recipient pulmonary function, with a particular focus on measuring percent-predicted forced 1-second expiratory volume (FEV1%) (24). They had 9,471 postoperative FEV1 and forced vital capacity (FVC) values from 509 adult transplant recipients, and performed a longitudinal temporal evaluation of FEV1% values for each patient. Mason and colleagues found that for both BLT and SLT patients, FEV1% typically peaked at 1 year after transplant. Forced 1-second expiratory volume increased from 50% in the immediate postoperative period to 55% at 1 year post-operatively in SLT recipients, and then gradually declined to 47% by three years. BLT recipients exhibited a similar trend but had higher overall FEV1 values at every time point (60% immediately post-transplant, 75% 1-year post-transplant, and 65% 3 years post-transplant). The authors also noted an increased mortality risk associated with decline in post-transplant FEV1 values in all recipients. Although patients undergoing either SLT or BLT exhibited increased risk of death with declining FEV1, this association in BLT recipients was notably tempered. The authors suggested that BLT may confer a protective effect on FEV1—and thus survival—likely as a function of providing recipients with enhanced pulmonary reserve. They recommended consideration of functional status in identifying which age groups would obtain maximal benefit from lung transplantation.

Pêgo-Fernandes also demonstrated relative improved pulmonary function (as measured by spirometry) among patients who underwent BLT (23). They performed a small, single-institution review of FVC and FEV1 data among lung transplant recipients between 2003 and 2006. Twenty-nine patients underwent transplant and were alive after the first postoperative year, and were thus included in analysis. Of these, 11 patients underwent SLT and 18 patients underwent BLT. All patients underwent spirometry pre-transplantation, and at 1-, 3-, 6-, 9-month, and 1-year intervals post-transplantation. Baseline characteristics of each cohort showed that patients who underwent BLT were younger but had significantly worse pre-transplant pulmonary function (mean FEV1 23.68 in BLT patients versus 44.11 in SLT, P<0.001). Similar to the findings demonstrated by Mason and colleagues, FEV1 and FVC peaked at 1-year post-transplantation for all transplant recipients. The BLT group had proportionally higher 1-year post-transplantation FEV1 values. The authors hypothesized that worse spirometry results among SLT patients could be attributed to hyperinflation or progression of the underlying disease in the native lung. However, the extremely small sample size of each cohort should be noted.

Pochettino observed improved pulmonary function and exercise tolerance in COPD recipients of bilateral transplantation (25). Similar to previous studies, they performed a single center retrospective study of 130 patients with emphysema from 1991–1999. Eighty-four patients underwent SLT and 46 patients underwent BLT. In addition to survival, the authors measured secondary outcomes of spirometry and 6-minute walk distances pre-operatively and at 3- to 6-month intervals post-operatively. While the authors prefer BLT (especially in younger patients) given their own institutional experience, they had utilized SLT on a more frequent basis due to scarcity of available donors. BLT was rarely utilized for recipients >60 years of age. Baseline FEV1, FVC, and 6-minute walk scores were similar between cohorts. At all post-transplantation time points during a 4-year observation period, BLT recipients exhibited higher FEV1 and FVC values compared to SLT, despite having similar baseline pulmonary function. Additionally, BLT patients had a higher mean 6-minute walk distance at all follow-up time points compared to SLT patients, with the difference ranging from 100 to 400 feet. It should be noted that the comparisons of spirometry values and exercise tolerance in this study were not adjusted for confounding characteristics. For example, with the authors favoring the use of SLT in older recipients, the BLT recipients were measurably younger (51.1 versus

56.2 years, P<0.0001). In this sense, the SLT cohort was preferentially burdened by a group of patients with more advanced age, and presumably more comorbidities and frailty. Further assessment of comorbidity or pre-transplant disease severity was not performed. The authors concluded with their preference for BLT in younger recipients due to the superior functional results and quality-of-life payoff the bilateral approach affords. The degree to which their a priori programmatic adoption of a BLT strategy for younger recipients created this appearance of improved function is impossible to measure.

Gerbase performed a combined prospective analysis of post-transplantation functional status and QOL (26). Focusing on spirometry and 6-minute walk distance, they prospectively enrolled 44 patients prior to lung transplantation. Fourteen (32%) eventually received SLT, while the remainder received BLT. Spirometry measurements and exercise assessment were performed before the transplant, as well as 6 and 12 months post-transplantation. Patients included in the report were followed for at least 2 years post-transplantation, raising some concerns about "survivor bias" and challenging the degree to which the result apply to patients on the waiting list. Although transplantation provided higher FEV1% predicted compared to baseline in all patients, this effect was dramatically lower among SLT recipients. At each time point over four years post-transplantation, SLT patients consistently had spirometry values at least 20% lower than spirometry scores of BLT recipients. 6MWT distances were not significantly different between cohorts, however.

Instead of spirometry, Genao utilized a comprehensive performance score (Karnofsky performance score, KPS) to gauge functional status in older lung transplant recipients (27). Genao wanted to characterize the long-term (1–5 years post-transplantation) trajectory of physical function, and subsequently analyze trends in older (>65 years) recipients of single and bilateral lung transplants. The authors performed a retrospective review of 4,805 patients listed in the UNOS registry between 2005 and 2009. Of these, 774 patients were at least 65 years of age, and 63% of this older subset received SLT. They began their analysis at 11 months post-transplantation based on the assumption that all patients would naturally undergo a postoperative period of disability and functional recovery within the first year after transplantation. KPS were assessed for all patients. The KPS was initially developed in the 1940s, and was a clinician-rated measure that estimated the patient's ability to conduct his or her

daily activities/self-care with none, some, or complete assistance. The score ranges from 0 to 100, and a score of 60 or less was traditionally associated with a higher risk for hospitalizations, the need for clinic visits, a serious functional decline, or mortality (28). The authors found that mean KPS scores at 1-year post-transplantation were higher than seen prior to transplantation for all recipients. One-year post-transplantation KPS was, on average, 2.6 points higher (on the scale of 100) for BLT than SLT recipients (P<0.0001). In subsequent years, there was an average 3.2 points decline for all patients, regardless of transplantation type. While BLT was associated with higher KPS post-transplantation, the authors noted that it was very rare for patients of either group to reach a level of disability predictive of poor outcomes (KPS ≤60) within the 5-year follow-up period. Thus, Genao and colleagues were unable to support the use of BLT in older recipients based on predicted KPS scores. There were important limitations to this study. The authors cautioned that conclusions regarding use of BLT *vs.* SLT based on their findings should be tempered, as they were unable to control for comorbidities or provider preference. Additionally, the study took into account the immediate perioperative functional decline associated with the recovery period of transplantation and included only patients were alive and had KPS scores after 11 months post-procedure. However, they did not discuss how they handled longitudinal measurement of KPS scores in patients who died after the 11-month cutoff. If QOL measurements were only taken from those who survived, there could be a survivor bias associated with the results. Additionally, the authors mention that the KPS is a clinician-based assessment, and is not a patient reported outcome instrument. Clinician assessment of a patient's QOL can vary from the individual patient's experience and the clinician might be biased when assigning such scores.

Post-transplantation QOL outcomes

For most patients with end-stage lung disease, lung transplantation cannot only provide a survival advantage, but can also influence dramatic changes in health-related QOL (HRQL). The most significant gains in HRQL are expected to be seen in physical health and functioning, and the greatest improvements are expected to occur early (within the first 6 months) after transplant (26). After 1 year, the risk of onset of BOS and the effect of other patient comorbidities can blunt the effect of transplantation on HRQL (29). Research into patient-centered outcomes

in the field of lung transplantation has received growing attention over recent years. However, the available literature on this topic is relatively lacking, and there are even fewer studies that attempt to examine the influence of transplantation type on QOL.

Certain cross-sectional studies have asserted a positive effect of BLT on HRQL measures. Anyanwu performed a European multicenter cross-sectional study of 255 lung transplant recipients (30). They administered the EuroQOL 5D (EQ5D) and visual analog scale (VAS) health-utility instruments to patients who received bilateral (n=79), single (n=106) and heart-lung (n=70) transplants. The EQ5D defines health quality in five dimensions: mobility, self-care, usual activities, pain and discomfort, and anxiety or depression (30,31). Survey takers can assign one of three labels to each dimension: no problem, moderate problem, or severe problem. Utility scores can then be assigned to each of these health states using regression analysis. The VAS allows participants to subjectively assess their own health on a scale of 0 to 100 (worst possible health to best possible health). In addition to stratifying results by transplant type, the authors repeated surveys at four different post-transplant time periods: 0–6, 7–18, 19–36, and >36 months. Problems in all five EQ5D domains in all time periods were more common among SLT patients than BLT patients. Those who received bilateral or combined heart-lung transplants had significantly higher EQ5D and VAS scores than their SLT counterparts in all time groups after 6 months (P=0.001). However, this study was limited by the lack of controlling for age and pre-transplant diagnosis.

The positive impact of BLT on HRQL was not demonstrated by all studies. Gerbase administered the St. George's Respiratory Questionnaire (SGRQ) and the VAS to 34 patients who had undergone SLT (n=14) or BLT (n=30) (26). The SGRQ primarily addresses three areas: respiratory symptoms, accomplishment of routine activities and disease impact on daily life (32). These patients were followed for at least 2 years (when the authors believed average onset of BOS occurs) and all data were collected prospectively. The authors noted that SGRQ and VAS scores were significantly improved after transplant compared to pre-transplant in both SLT and BLT groups. However, post-transplant, there was no significant differences in QOL scores between SLT and BLT groups. Scores were also independent of the underlying disease that led to transplantation. As described in the previous section, the authors also collected spirometric and 6MWT

data and found that the post-transplantation improvement in FEV1% predicted scores were significantly less in SLT versus BLT recipients. 6MWTs were comparable between cohorts. The authors suggested that pulmonary function had limited influence on objective and subjective parameters of patient health-related QOL.

Copeland prospectively studied QOL measures in patients who were 1-year post-transplantation (33). They utilized a pre-existing study cohort of 131 lung transplant patients who were already prospectively enrolled in a cytomegalovirus (CMV) prevention trial. To obtain data on physical and mental health QOL measures, they surveyed these patients immediately pre-transplant, as well as 3, 6, 9, and 12 months after transplant using the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36). As a part of the SF-36, scores are assigned to develop the Physical Component Survey and Mental Component Survey (34). These scores were followed longitudinally over the first post-transplant year. The authors used linear mixed modeling for repeated measures of QOL scores, which relied on any data collected at any time point to longitudinally estimate scores. This approach was used because the authors anticipated missing data from loss to follow-up or death. Over this time period, Physical Component Survey scores rose by an average of 10.9 points from baseline (P<0.0001), reaching a level close to the average US population score. Mental Component Survey scores did not exhibit a significantly dramatic rise. When stratified by transplant type, bilateral operations did not confer a significant advantage in gains in physical component scores over single lung transplant. Given that the functional outcome benefit conferred by bilateral procedures has been shown to be greater in the long term (>1 year), it would be interesting to see if there would be a clinically important difference in QOL scores if they were longitudinally followed over a longer period of time.

Associations between procedure type and chronic rejection

BOS syndrome after lung transplantation represents chronic allograft rejection and dysfunction (35). BOS syndrome is defined as a progressive airflow obstruction with deterioration of graft function, and affects up to 60% of lung transplant recipients who survive 5 years post-transplantation (36). The mean time between transplant and diagnosis is approximately 16–20 months (35). It has been characterized by a continuous deterioration in

FEV1% predicted, and can be pathologically confirmed by the presence of intraluminal fibromyxoid granulation tissue and extensive eosinophilic infiltrates on transbronchial biopsy (37).

BOS has been linked to many poor outcomes in transplant recipients, with increased mortality risk and an association with decreased functional and HRQL outcomes (35). While the exact physiologic mechanism behind the development of BOS is unknown, multiple studies have reported SLT as a risk factor for development of BOS (26,38,39). Neurohr and colleagues performed an institutional review of their lung transplant database and compared 46 SLT and 30 BLT recipients with a diagnosis of IPF (38). SLT was found to be a predictor for occurrence of BOS ≥ stage 1. Another small institutional study performed by Gerbase, who noted that risk of BOS development at 24 months post-transplant was more than two times higher in SLT recipients (RR 2.86; 95% CI, 1.22–6.67) (26). Hadjiliadis found SLT to be independently associated with BOS occurrence after transplantation (39). In their single center retrospective study of 225 transplant recipients, they found an overall incidence of BOS to be 41.3% at a median time of 4.2 years since transplant. After controlling for other patient comorbidities and characteristics, SLT was found to be significantly associated with BOS onset in multivariable regression analysis. Other variables including transplant center, recipient age, and end-stage lung disease diagnosis were not associated with risk of BOS development. As diagnosis of BOS depends on the decline in FEV1%, it makes sense that SLT patients are at increased risk of BOS development. Unlike BLT patients, SLT patients still have a diseased native lung, and its deterioration over time contributes to their overall FEV1%. Thus, in a hypothetical situation where the recipient risk factors and donor lungs are equal, an SLT patient may have a higher baseline risk for meeting the threshold of a BOS diagnosis compared to a BLT patient simply due to native lung dysfunction.

Transplant center, recipient age, re-sapient diagnosis, gender, acute rejection score and number of bronchoscopies in the first 6 months had no effect on the risk of BOS development.

Not all studies have demonstrated the same association between transplantation type and onset of BOS. In a much larger UNOS database analysis of 2,260 lung transplant recipients with primary diagnosis of COPD, Meyer and colleagues did not observe a difference in BOS incidence between SLT and BLT cohorts over the three-year follow-up period (4). Given the enormous morbidity and mortality burden that BOS imposes on lung transplant recipients, further research is warranted to investigate the physiologic mechanism of BOS and any possible link there may be to transplantation type.

Ethical considerations

Much of the debate surrounding use of BLT or SLT stems from the ethical challenge of how best to make use of a limited resource: donor lungs. The persistent ethical dilemma surrounding lung transplantation is whether the possible broader societal benefits of splitting a pair of donor lungs and thus reducing wait list time and wait list mortality outweighs the cost to the individual recipient to forego BLT. Several institutions, including our own, routinely use BLT for most lung transplant recipients (10). This brings to head the ethical dilemma posed by BLT, and challenges the reader to decide whether increased individual benefit is worth the societal cost of fewer patients transplanted.

Several groups have found innovative methods to determine the opportunity cost of providing bilateral operations. Anyanwu examined lung donors reported to the United Kingdom Cardiothoracic Transplant Audit between 1995 and 1998 for whom both lungs were utilized (40). They examined survival, rejection, and infection of donor recipients of these lungs to make comparisons between single lung and bilateral lung recipients. One-year graft survival for single lung and bilateral lung blocks were similar (65% vs. 71%). Of donor blocks that went to SLT recipients, both grafts were functioning in 44% of donor blocks, both grafts failed in 14% of donor blocks, and one of the two lungs failed in 42% of donor blocks. The authors estimated that splitting a lung block for SLT produced 1.8 survivors per donor block at 1-year post-transplant. One of the weaknesses in their study is that they did not stratify by clinical diagnosis, and they even included a large number of patients with CF and those undergoing re-transplantation in their analysis. In another study, Anyanwu and colleagues examined cost-effectiveness of transplantation versus medical therapy, and included additional comparisons of SLT and BLT (41). They determined that over a theoretical 15-year period, transplantation (compared to remaining on the waitlist with medical therapy) provided 2.1 and 3.3 quality-adjusted life-years (QALY) for SLT and BLT, respectively. The average cost of medical therapy for those not receiving a transplant during this period of time was $73,564. The costs of SLT and BLT were $176,640 and $180,528, respectively. Costs per each QALY gained were

$48,241 for SLT and $32,803 for BLT. Based on the cost per QALY gained, the authors concluded that SLT was the least cost-effective form of therapy for patients with end stage lung disease. However, they noted that they were unable to quantify the additional societal gain that would come from the SLT's ability to treat more patients. Also, it may be possible that the cost to society would be more accurately measured in total cost and not cost per QALY. A plan of more single lung transplant operations will lead to more operations in general and more patients on the very expensive post-transplant medications. The broad use of single rather than bilateral operations could greatly increase the total cost of lung transplantation programs to a society or a payer.

Wang took into account not only the ethical challenges in offering one versus two lungs, but the effect of remaining on the transplant waiting list longer with the hopes to undergo bilateral transplantation (42). Utilizing data obtained from national UK transplant database, they performed a sequentially stratified proportional hazards model on 1,211 adult lung transplant patients to address the following question: "should I accept SLT if offered or should I remain on the waiting list in the hope that I will be offered BLT in the future." They found that in patients with pulmonary fibrosis, SLT was associated with a significant reduction in mortality hazard relative to waiting for BLT (HR 0.81, 95% CI, 0.68–0.97, P=0.021). They concluded that for pulmonary fibrosis patients, accepting SLT outweighed remaining on the transplant list for a BLT by minimizing the high pre-transplant risk of death. There was no such benefit demonstrated in accepting SLT for patients with COPD, however. Munson and colleagues reached a different conclusion (43). They created a simulation of a lung transplant waitlist using actual post LAS implementation UNOS registry data to define waitlist size, donor frequency, waitlist mortality risk, and disease- and procedure-specific post-transplantation survival. They aimed to determine post-transplant survival associated with BLT versus SLT in the COPD population. They determined that SLT always increased the number of patients transplanted, without significant reductions in total post-transplant survival. A theoretical policy of uniform use of SLT in their model resulted in an absolute reduction in the risk of waitlist mortality of 4.2% among all listed patients. However, they noted that this pattern may not be reproducible once geographic donor variations are accounted for and could not be compared to other transplant disease indications.

While the common ethical argument suggests that SLT may provide greater societal benefit by maximizing utilization of the existing donor pool, this may not be an accurate depiction. One study used the UNOS registry to study lung block utilization in all SLTs performed between 1987 and 2011. There were 7,232 unique SLT donors identified. Of these donors, only 3,129 (43%) had both lungs used for SLT. The authors reported that more than 200 potential donor lungs went unused annually since 2005. Donor factors associated with the harvest and use of only one lung included type B/AB blood group, lower BSA, lower pO_2, pulmonary infection, extended criteria donor status, and traumatic brain injury or anoxia as cause of death. This study challenged one of the long-standing utilitarian arguments in favor of SLT (44).

At our own institution, there is a greater preference to perform BLT in part due to the prevailing notion that two lungs provide patients with greater physiologic reserve (10). Given this assumption, we often use what might be considered "marginal" donor lungs for BLT for patients—donor organs that would otherwise might be wasted if considered individually in single lung blocks and thus declined. Similarly, in geographic situations in which donor lungs are not considered by a large number of programs, the ability to use two lungs might allow a physically small donor to provide lung transplantation for a much larger recipient. Therefore, it is possible that there is an occasional situation in which the use of donor lungs is "both or none". In this sense, BLT may expand donor lung utilization. Further research into the use of marginal donors/extended donor criteria and subsequent impact on lung resource allocation will be necessary to clarify the nuances in the BLT *vs.* SLT.

The ethical considerations of BLT *vs.* SLT encourage surgeons and institutions to determine priorities: optimizing total number of potential recipients who get transplanted or enhancing post-transplant survival. It is likely that these two goals might be at odds with each other. While adopting SLT will definitely increase the number transplanted, this may come at the expense of post-transplant long-term survival. Implementation of the LAS on transplant lung allocation practices aims to reduce transplant waitlist mortality. However, much of the ethics surrounding the debate will be expressed by institutional preference and practice.

The native lung: potential complications and risk of cancer

One special consideration for the use of SLT is the risk

of potential complications in the native lung. Native lungs already have diminished lung function secondary to underlying disease process, and the use of SLT can potentially impose the additional complications. Venuta and colleagues described their experience in native lung complications in an institutional review (45). From 1991–1997, they reviewed 35 patients who received SLT, of which 11 patients experienced an early (<6 weeks) native lung complication. These complications included overinflation, pneumothorax, hemothorax, pneumonia, invasive aspergillosis, and active tuberculosis (which was present at time of initial transplant). These patients underwent a mix of medical and surgical therapy, with 3 patients receiving an operation. Mortality was still high, with 6 of these patients dying within 6 months. King and colleagues also described their institutional experience with SLT, and also studied outcomes of pneumonectomy for native lungs that experienced complications (46). In 180 single lung transplants performed from 1998–2008, 25 patients (14%) experienced significant native lung complications. Of these, 11 patients went on to receive a pneumonectomy for non-small cell lung cancer (NSCLC), aspergilloma, bronchopleural fistula, and recurrent infection. Complication rates after receiving pneumonectomy were high (36.4%), but there was no in-hospital mortality. Additionally, when comparing patients who received a pneumonectomy for a complication to those who did not experience a complication, there was no statistically significant difference in median survival (4.3 *vs.* 5.1 years). Thus, King concluded that while native lung complications impose serious morbidity and mortality, pneumonectomy could provide an acceptable solution.

While these studies highlight the potentially serious morbidity and mortality of native lung complications, certain points should be noted. First, the data presented in these studies are of limited sample size and are relatively outdated. The perioperative management of transplant patients has undergone substantial improvement over the years, calling into question whether high volume SLT centers today would experience the high rate of native lung complications. While early recognition and management of native lung complications is important, the possibility of developing a native lung complication does not necessarily preclude the use of SLT.

One additional concern regarding SLT is the risk of cancer development in the native lung. Citing increased risk associated with long-term chronic lung disease, possible recipient smoking history, increased age, and potential

adverse effects of immunotherapy, one review documented a 9% prevalence of primary lung cancer found in native lungs after SLT (47). Olland and colleagues acknowledged that surgical resection for early stage NSCLC of the native lung should be pursued when possible, but the effects of chronic lung disease and immunosuppression may make surgery more challenging than when compared to a non-transplanted patient (47). Nevertheless, the benefits of a SLT may still outweigh the risks of a BLT in a patient with high LAS. Appropriate resource utilization should be geared towards thorough and aggressive surveillance for malignancy in high-risk SLT patients.

Future directions: SBLT?

As a possible compromise between SLT and BLT, Hartwig and colleagues have proposed SBLT for high-risk patients with ILD (48). To mitigate perioperative morbidity and mortality risk and to preserve the observed long-term benefit of BLT, these authors proposed utilization of SLT in some recipients and then relisting them for a subsequent contralateral SLT at a future date. Typically, an institution using this strategy will list individuals deemed to have higher perioperative risk (by age or comorbidity) to undergo SLT. After transplantation, these patients are reviewed for re-listing and all individuals who were noted to have acceptably low perioperative complications and reasonable functional status were considered. Re-listing for contralateral transplant was performed as soon as was clinically appropriate (as determined by adequate functional recovery and no presence of infection or rejection). The authors performed a matched cohort analysis with a primary outcome of survival. Twelve patients underwent SBLT, and matches were selected in a 1:2:2 ratio from SLT and BLT recipients with ILD and similar LAS score. When comparing characteristics between the first and second stages of the SBLT procedure, there were no significant differences between donor characteristics. LASs were significantly higher in the first stage compared to the second stage (48.6 *vs.* 24.5, P<0.01). When comparing between matched cases, the authors found no significant differences in survival. The authors thus proposed SBLT as an alternative to SLT and BLT. The concept of SBLT is intriguing, but the strategy itself is fraught with potential complications and ethical challenges. The authors noted that the staged bilateral option exposes patients to two operations, and there may be a pool of individuals who sustain the risks of a second procedure when they could

have done reasonably well with just the initial single lung transplant. Additionally, the existing knowledge on the immunologic consequences of receiving a second lung from a separate donor is relatively limited. It is unclear whether these patients will be at greater or reduced risk for developing lung allograft dysfunction long-term. From an ethical perspective, it is unclear whether SBLT truly results in a better redistribution of a limited resource. While more lungs would be available for use if individuals underwent a unilateral first stage operation instead of a BLT, many would ultimately reappear on the waitlist. It is unclear whether the second donor lung would achieve more benefit as a second implant for a staged procedure recipient or being utilized for a new patient who has never undergone transplantation. Although several important questions regarding use of SBLT exist, it still remains a controversial option and further investigation into the subject may be warranted.

Conclusions

BLT has grown in utilization among transplant centers nationally, and presents a useful option for patients with a variety of end-stage lung disease diagnoses. The increased adoption of BLT is likely reflective of increased comfort in practice among transplant surgeons and recognition of benefits measured by long-term survival and improvements in functional and QOL outcomes. However, much of the literature that examines the use of BLT versus SLT is conflicting, and the clinical picture is further nuanced by disease indication, age of recipient, donor lung quality and patient disease severity. Although it is our institutional preference to utilize BLT in our patient population when possible, we cannot recommend one procedure type over another given the lack of high quality evidence. Transplantation type will continue to be determined on an individual basis. The current clinical picture of transplantation in the post-LAS era is certainly different than before, but much of the existing data available is not yet reflective of this change. There will likely never be a randomized trial to clarify the respective roles of BLT and SLT. However, further large database analyses and prospective observational studies will be instrumental to bring clarity to this debate.

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Footnote

References

1. Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary fibrosis. N Engl J Med 1986;314:1140-5.
2. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.
3. Puri V, Patterson GA. Adult lung transplantation: technical considerations. Semin Thorac Cardiovasc Surg 2008;20:152-64.
4. Meyer DM, Bennett LE, Novick RJ, et al. Single vs bilateral, sequential lung transplantation for end-stage emphysema: influence of recipient age on survival and secondary end-points. J Heart Lung Transplant 2001;20:935-41.
5. Meyer DM, Edwards LB, Torres F, et al. Impact of recipient age and procedure type on survival after lung transplantation for pulmonary fibrosis. Ann Thorac Surg 2005;79:950-7.
6. Puri V, Patterson GA, Meyers BF. Single versus bilateral lung transplantation: do guidelines exist? Thorac Surg Clin 2015;25:47-54.
7. Chambers DC, Yusen RD, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-fourth Adult Lung and Heart-Lung Transplantation Report- 2017; Focus Theme: Allograft ischemic time. J Heart Lung Transplant 2017;36:1047-59.
8. Aigner C, Klepetko W. Bilateral lung transplantation. Oper Tech Thorac Cardiovasc Surg 2012;17:181-93.
9. Thabut G, Christied JD, Ravaud P. Survival after bilateral versus single lung transplantation for patients with chronic obstructive pulmonary disease: a retrospective analysis of registry data. Lancet 2008;371:744-51.
10. Cassivi SD, Meyers BF, Battafarano RJ, et al. Thirteen-year experience in lung transplantation for emphysema. Ann Thorac Surg 2002;74:1663-9; discussion 1669-70.
11. Bennett DT, Zamora M, Reece B, et al. Continued utility of single-lung transplantation in select populations:

chronic obstructive pulmonary disease. Ann Thorac Surg 2015;100:437-42.

12. Schaffer JM, Singh SK, Reitz BA, et al. Single- vs double-lung transplantation in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis since the implementation of lung allocation based on medical need. JAMA 2015;313:936-48.

13. Lane CR, Tonelli AR. Lung transplantation in chronic obstructive pulmonary disease: patient selection and special considerations. Int J Chron Obstruct Pulmon Dis 2015;10:2137-46.

14. Kistler KD, Nalysnk L, Rotella P, et al. Lung transplantation in idiopathic pulmonary fibrosis: a systematic review of the literature. BMC Pulm Med 2014;14:139.

15. Mapel DW, Hunt WC, Utton R, et al. Idiopathic pulmonary fibrosis: survival in population based and hospital based cohorts. Thorax 1998;53:469-76.

16. Raghu G, Collard HR, Egan JJ, et al. Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788-824.

17. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014- An update from the Pulmonary Transplantatio council for the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.

18. Mason DP, Brizzio ME, Alster JM, et al. Lung transplantation for idiopathic pulmonary fibrosis. Ann Thorac Surg 2007;84:1121-8.

19. Weiss ES, Allen JG, Merlo C, et al. Survival after single versus bilateral lung transplantation for high-risk patients with pulmonary fibrosis. Ann Thorac Surg 2009;88:1616-25; discussion 1625-6.

20. Force SD, Kilgo P, Nejahr DC, et al. Bilateral lung transplantation offers better long-term survival compared with single-lung transplantation, for younger patients with idiopathic pulmonary fibrosis. Ann Thorac Surg 2011;91:244-9.

21. Chauhan D, Karanam AB, Merlo A, et al. Post-transplant survival in idiopathic pulmonary fibrosis patients concurrently listed for single and double lung transplantation. J Heart Lung Transplant 2016;35:657-60.

22. Nwakanma LU, Simpkins CE, Williams JA, et al. Impact of bilateral versus single lung transplantation on survival in recipients 60 years of age and older: Analysis of United Network for Organ Sharing database. J Thorac Cardiovasc Surg 2007;133:541-7.

23. Pêgo-Fernandes PM, Abrão FC, Fernandes FL, et al. Spirometric assessment of lung transplant patients: one year follow-up. Clinics (Sao Paulo) 2009;64:519-25.

24. Mason DP, Rajeswaran J, Li L, et al. Effect of changes in postoperative spirometery on survival after lung transplantation. J Thorac Cardiovasc Surg 2012;144:197-203.

25. Pochettino A, Kotloff RM, Rosengard BR, et al. Bilateral versus single lung transplantation for chronic obstructive pulmonary disease: intermediate-term results. Ann Thorac Surg 2000;70:1813-8; discussion 1818-9.

26. Gerbase MW, Spiliopoulos A, Rochat T, et al. Health-related quality of life following single or bilateral lung transplantation. Chest 2005;128:1371-8.

27. Genao L, Whitson HE, Zaas D, et al. Functional status after lung transplantation in older adults in the post-allocation score era. Am J Transplant 2013;13:157-66.

28. Crooks V, Waller S, Smith T, et al. The use of the Karnofsky Performance Scale in determining outcomes and risk in geriatric outpatients. J Gerontol 1991;46:M139-44.

29. Vermeulen KM, Ourwens JP, van der Bij W, et al. Long-term quality of life in patients surviving at least 55 months after lung transplantation. Gen Hosp Psychiatry 2003;25:95-102.

30. Anyanwu AC, McQuire A, Rogers C, et al. Assessment of quality of life in lung transplantation using a simple generic tool. Thorax 2001;56:218-22.

31. EuroQol Group. EuroQol: a new facility for the measurement of health related quality of life. Health Policy 1990;16:199-208.

32. Jones PW, Quirk FH, Baveystock CM. The St. George's Respiratory Questionnaire. Respir Med 1991;85 Suppl B:25-31; discussion 33-7.

33. Finlen Copeland CA, Vock DM, Pieper K, et al. Impact of lung transplantation on recipient quality of life. Chest 2013;143:744-50.

34. Ware JE, Gandek B. Overview of the SF-36 health survey and the International Quality of Life Assessment (IQOLA) project. J Clin Epidemiol 1998;51:903-12.

35. Al-Githmi I, Batawil N, Shigemura N, et al. Bronchiolitis obliterans following lung transplantation. Eur J Cardiothorac Surg 2006;30:846-51.

36. Boehler A, Kesten S, Wedder W, et al. Bronchiolitis obliterans after lung transplantation. Chest 1998 114:1411-26.

37. Burke CM, Theodore J, Dawkin KD. Post-transplant

obliterative bronchiolitis and other late lung sequelae in heart-lung transplantation. Chest 1984;86:824-9.

38. Neurohr C, Huppmann P, Thum D, et al. Potential functional and survival benefit of double over single lung transplantation for selected patients with idiopathic pulmonary fibrosis. Transpl Int 2010;23:887-96.

39. Hadjiliadis D, Angel LF. Controversies in lung transplantation: are two lungs better than one? Semin Respir Crit Care Med 2006;27:561-6.

40. Anyanwu AC, Rogers CA, Murday AJ. Does Splitting the lung block into two single lung grafts equate to doubling the societal benefit from bilateral lung donors? Comparisons between two single versus one bilateral lung transplant. Transpl Int 2000;13:S201-2.

41. Anyanwu AC, McGuire A, Rogers CA, et al. An economic evaluation of lung transplantation. J Thorac Cardiovasc Surg 2002;123:411-8; discussion 418-20.

42. Wang Q, Rogers CA, Bonser RS, et al. Assessing the benefit of accepting a single lung offer now compared with waiting for a subsequent double lung offer. Transplantation 2011;91:921-6.

43. Munson JC, Christie JD, Halpern SD. The societal impact of single versus bilateral lung transplantation for chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2011;184:1282-8.

44. Speicher PJ, Ganapathi AM, Englum BR, et al. Single lung transplantation in the United States: What happens to the other lung? J Heart Lung Transplant 2015;34:36-42.

45. Venuta F, Boehler A, Rendina EA, et al. Complications in the native lung after single lung transplantation. Eur J Cardiothorac Surg 1999;16:54-8.

46. King CS, Kandhar S, Burton N, et al. Native lung complications in single-lung transplant recipients and the role of pneumonectomy. J Heart Lung Transplant 2009;28:851-6.

47. Olland ABM, Falcoz PE, Santelmo N, et al. Primary lung cancer in lung transplant recipients. Ann Thorac Surg 2014;98:362-71.

48. Hartwig MG, Ganapathi A, Osho AA, et al. Staging of bilateral lung transplantation for high-risk patients with interstitial lung disease: one lung at a time. Am J Transplant 2016;16:3270-7.

Single-lung and double-lung transplantation: technique and tips

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Abstract: The first successful single-lung and double-lung transplantations were performed in the eighties. Since then both surgical and anesthesiological management have improved. The aim of this paper is to describe the surgical technique of lung transplantation: from the anesthesiological preparation, to the explantation and implantation of the lung grafts, and the preparation of the donor lungs. We will also describe the main surgical complications after lung transplantation and their management. Each step of the surgical procedure will be illustrated with photos and videos.

Keywords: Double-lung transplantation; single-lung transplantation; surgical technique; bronchial anastomosis

Introduction

After the first successful single-lung transplantation in 1983, the Toronto team described the first successful double-lung transplantation in 1988 (1,2). The lungs were implanted "en-bloc" through a median sternotomy, three anastomoses were performed: one tracheal, one on the pulmonary artery (PA) and one on the left atrium. This procedure was proved to be technically demanding and likely to lead to impaired airway anastomosis healing (3).

It has been therefore abandoned in favor of the bilateral sequential technique where one single lung transplantation is performed after the other, with or without associated cardiopulmonary bypass (CPB), as described by the Foch and St Louis teams (4-6).

Anesthesiologic preparation (*Figure 1*)

The anesthetic preparation of the recipient is the first step of the surgery, and needs a well-trained team, as well as specific devices.

In addition to the usual induction tools and double-lumen endotracheal tube (Carlens), the anesthesiologist should prepare an autologous blood recovery system such as Cell Saver®, a Swan-Ganz catheter for monitoring the pulmonary arterial pressure, cardiac index and SVO_2. A deep venous catheter is placed in a jugular vein (4 lines), as well as a radial arterial line for monitoring of the blood pressure, and two peripheral venous catheters. Depending on the experience of the anesthesiologist a probe for trans-esophageal echocardiography is placed, or a nasogastric tube if not. If cardio-pulmonary bypass is expected to be required during surgery, the Swan-Ganz catheter is not mandatory and visual monitoring of heart function can be performed by trans-esophageal echography exclusively. A bladder catheter is also placed, allowing monitoring of diuresis and body temperature.

A defibrillator should be present in the operating theatre, as well as the material necessary to put a chest tube in case of pneumothorax during the induction (especially for COPD

Figure 1 Anesthesiologic preparation. 1, double-lumen tube; 2, central venous catheter; 3, Swan-Ganz probe; 4, invasive blood pressure/arterial catheter.

Figure 2 Installation. Arms spread.

Figure 3 Clamshell incision. (A) Cutaneous incision; (B) surgical view after opening the chest.

patients). Adequate amount of blood is ordered depending on the expected difficulty with the surgery [patient history and potential extracorporeal membrane oxygenation (ECMO)] and the medications taken by the recipient.

The patient is put to sleep only after the procurement team has accepted the lungs. The thoracic surgeon should have arrived by this point. If a patient has pre-operative pulmonary hypertension, a peripheral venoarterial ECMO may be necessary before induction of anesthesia. If not, the team must stay vigilant and be prepared to place the ECMO at any time for hemodynamic assistance in emergency conditions.

The whole preparation takes from one to two hours and must be planned during the initial organization of the lung transplantation.

Installation and surgical approach

The patient is placed either with his arms spread, or

crossed above his head (*Figure 2*). Initially a bilateral anterior thoracotomy in the fourth or fifth inter-costal space was performed, with an additional transversal sternotomy, realizing the clamshell incision. Because of the complications with this approach, such as pseudo-arthrosis, sternal infection, alteration of respiratory mechanics or dysfunction of the phrenic nerves, bilateral thoracotomy without sternal section is now performed whenever possible (*Figures 3* and *4A*). While the anterior thoracotomy is performed, the sub-cutaneous and breast tissue can be fixed upward to the operative field in case it is too voluminous (*Figure 4B*).

A clamshell approach is performed at the beginning of the lung transplant when a shrunken surgical field exposure is expected, as in patients with severe pulmonary fibrosis. Otherwise, it can be performed during the procedure if the patient becomes hemodynamically unstable, if unexpected pleural adhesions are found or hilum dissection is difficult. The need for ECMO does not necessarily require to split the sternum, as the surgeon has access to the descending aorta through the left thoracotomy, and the right atrium on the other side for cannulation.

Other approaches have been advocated:

❖ Lateral or posterolateral thoracotomy when difficult dissection is expected. But the patient then needs to be positioned a second time for the other side. This approach was used in the 2000's for
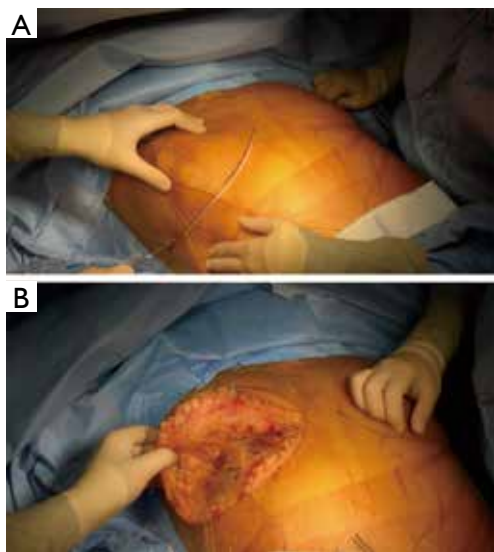
Figure 4 Double anterior thoracotomy. (A) Cutaneous incision. The right side is opened first; (B) breast tissue is pulled upward.

Video 1. ...ssection

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Figure 5 Lung dissection. The lung is gently divided from the mediastinum. The main PA and the mediastinal artery are dissected and encircled with loops, as well as the upper and middle lobes veins. The inferior vein is not seen on the video. Before explantation, a clamping test on the PA is performed, while the anesthesiologist checks hemodynamic tolerance and possible rise in pulmonary arterial pressures (8).
Available online: http://www.asvide.com/article/view/24494

single lung transplantation;
❖ Median sternotomy when no pleural adhesions are expected and probability of central CPB is high, in primary pulmonary hypertension for example;
❖ Video-assisted anterior thoracotomy has been described by the Hanover team (7).

Surgical technique

Anesthesia begins after the procurement team has confirmed the acceptability of the donor lungs. From this moment, everything must be done to shorten the cold ischemic time as much as possible. The native lung and the hilum are dissected before the procurement team arrives. The PA clamping test is done as well at that point. Explantation of the lung on the other hand, is typically done only when the donor lungs are on site.

The first side implanted depends on the pre-operative examinations. If there is no dominant lung in the recipient, the first lung implanted is usually the right one. Because of anatomy the implantation is usually easier and quicker on this side. Bilateral lung transplantation is the association of two single lung transplantations.

We will describe the right lung transplantation in detail.

Lung dissection

The first step is to free the native lung from the parietal pleura. In case of history of lung surgery, pneumothorax and chest tube, pleural symphysis or suppurative and chronic infectious lung disease, it can be challenging.

Single-lung ventilation should be avoided as long as possible, or associated with clamping of the PA, to prevent shunt and hypoxemia. Traction on the hilum should also be avoided, as well as lung injury, to prevent bleeding and hemodynamic instability. Special care should be given in order to avoid injury to any recurrent or phrenic nerve.

The aim of hilar dissection is to individualize each structure (bronchus, PA and left atrium) in order to be able to clamp these structures to perform safely the anastomoses distally from the clamp (*Figure 5*).

The pulmonary ligament is divided until the inferior pulmonary vein is reached. The veins are then freed from the surrounding pleura, which is pushed towards the lung, for better visualization and section of the adherences. The PA is then divided up until its first branch. A loop encircling the superior pulmonary vein first and then pulled downward can be helpful for exposure.

The veins are also encircled with loops and gently pulled, allowing the surgeon to have a good view on the pericardium, which is then opened with drainage of clear pericardial fluid. The easiest opening spot is located on the anterior and inferior side of the inferior vein. A dissector can be used to help completing the so-called pericardial window while dividing the pericardium around the whole

Figure 6 Preparation of the graft. The bronchus is cut one ring above the secondary carina to prevent ischemic necrosis. The artery is cut to avoid excessive length and later plicature of the anastomosis. The atrium cuff is made as regular as possible. All the removed pieces of the vascular structures are kept for eventual reparation of anastomotic leak (9).
Available online: http://www.asvide.com/article/view/24495

hilum. Careful coagulation of vessels coming from the sub-carinal space should be done while freeing the upper part of the upper pulmonary vein. The section of the posterior part of the pericardium is performed in regard of the Haller pericardial sinus, while the veins are pulled upward.

The phrenic nerve should be carefully preserved during the whole procedure; it is left a few millimeters above the pericardial opening. The anterior edges of the pericardial window are suspended using stiches to facilitate the exposure of the hilum, and avoid incidental phrenic nerve injury.

Once the veins and the left atrium have been freed from all pericardial adherences, an extra length of atrial wall can be gained by dissecting the interatrial groove, according to the Sondergaard maneuver, to facilitate lateral clamping of the atrium. The dissection begins in the sulcus, just behind the connection to the superior vena cava with the right atrium, and is continued caudally where the sulcus becomes hardly noticeable.

The PA is freed from the pericardium by section of the fascias between them, going further than the vena cava, which is retracted carefully to avoid trauma of the phrenic nerve. The dissection is performed until the beginning of the mediastinal artery can be seen, as the PA clamping must be performed centrally. Sometimes when the first segmental artery of the right upper lobe originates early from the right PA, the later may be approached and controlled in the Theile pericardial sinus, in-between the superior vena cava

and the ascending aorta.

Once all the vascular elements of the hilum are under control, a clamping test is performed on the PA. It allows the surgeon to check if the contralateral lung and the heart will provide sufficient and stable oxygenation and hemodynamics throughout the explantation and implantation of the other lung.

Saturation, blood pressure, pulmonary blood pressure and impact on the heart function (trans-esophageal echography) are monitored during the test. Though no specific time can be given, we usually perform a clamping test for ten minutes before explantation.

Active and repeated bronchial aspirations, pressure-controlled ventilation (volume 5 mL/kg, PEEP 5 cmH$_2$O), administration of nitric oxide (increase of pulmonary arterial pressure), can improve unique lung ventilation while the contralateral lung is clamped.

While the lead surgeon explant the lung and prepare the hilum for the implantation, the procurement team prepares the lung graft (*Figure 6*).

The lungs are preserved inflated either at 4 °C in three sterile bags containing respectively the preservation fluid, sterile physiological serum, and air or with a portable device such as the Organ care system (OCS, Transmedics™) for normothermic preservation (*Figure 7*). The lungs can be split either after the procurement in the donor hospital or immediately before the transplantation in the recipient hospital, depending on the team's policy or the use of the OCS. The division of the two lungs is performed first on the left atrium in the middle of the posterior wall, than on the artery at the bifurcation, and finally on the trachea with a stapling device, leaving the right main bronchus and the trachea on one side, and the left main bronchus on the other. Retrograde flush via the pulmonary veins, useful to eliminate the last clots present in the graft, is performed as well at the site of procurement or prior to transplantation. The preservation solution is sampled routinely for microbiological analysis.

The preparation of the graft hilum has several goals:
- ❖ Resection of structures in excess: pericardium, fat, azygos vein, aortic arch, vena cava;
- ❖ Cutting the bronchus as short as possible. The donor bronchus is shortened with only one cartilage ring remaining proximal to the bifurcation of the upper lobe bronchus, or even at the level of the secondary carina, to prevent impaired bronchial anastomotic healing;
- ❖ Leave sufficient peribronchial fatty tissue to wrap the bronchial anastomosis once completed;
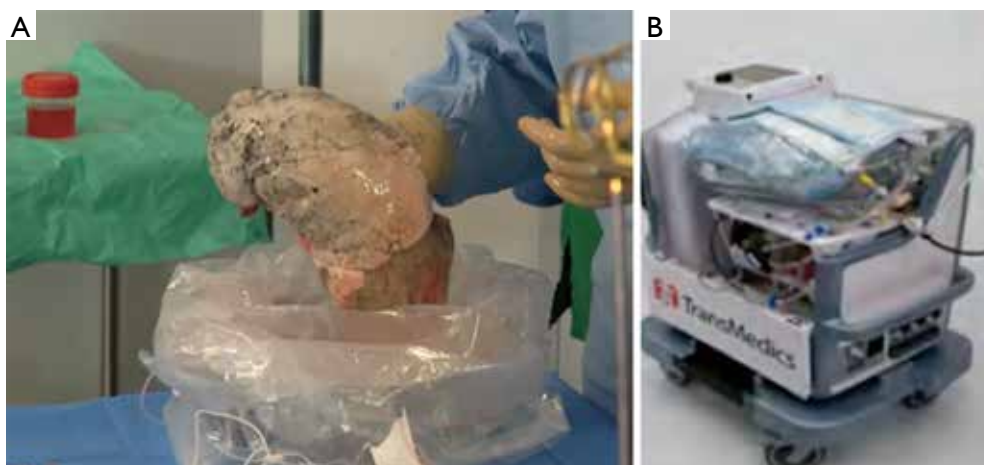
Figure 7 Preservation of the lung. (A) The graft is preserved at 4 ℃ in three bags containing respectively cold preservation fluid, cold physiological serum and ice, and air. The nurse opens the first bag, and the surgeon takes under sterile conditions the second bag inside. (B) Preservation with an OCS device (Transmedics®, Andover, MA, USA).

Video 3. Expla[ntation of] the native lung

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Figure 8 Explantation of the native lung. The vessels, pulmonary artery and pulmonary veins, are cut with a stapling device first. The bronchus is seen afterward, and opened with a sharp bistouri. From this moment the suction device must be changed, and the cell-saver cannot be used any longer until the bronchial anastomosis is done. The last adherences are cut, the native lung is removed from the chest cavity and sent for pathological examination (10). Available online: http://www.asvide.com/article/view/24496

❖ Prepare an artery not too long, in order to avoid later obstructive plication;

❖ Conservation of the resected pieces of veins and artery in preservation fluid, to be used as patches to manage potential vascular anastomotic problems later during the procedure.

Explantation of the native lung should be performed after the arrival of the procurement team.

At this point, hilar dissection has already been done and the following steps therefore go fast. Usually the veins are sectioned first with a stapling device, followed by the artery. On the right side, the mediastinal artery is often stapled separately from the main PA. Vessels stumps should be left as long as possible to facilitate the anastomoses (*Figure 8*).

The bronchus is then divided above the origin for the upper lobe bronchus. Careful hemostasis is performed. From this point, the surgical field is "contaminated" and the Cell Saver device to recover red blood cells can no longer be used until the bronchus is closed.

The PA is gently pulled to allow the surgeon to put a clamp on the artery as far proximal behind the superior caval vein as possible. If a Swan-Ganz probe has been placed, the surgeon should check its position and if the catheter is in the clamping area, withdraw it.

The same maneuver is performed on the left atrium, to test the feasibility and safety of later clamping.

The recipient bronchus is prepared centrally while avoiding denuding it beyond the level of the planned anastomosis. This contributes to prevent ischemia of the bronchial anastomosis, even if the main risk factor for this complication lies at the level of the donor bronchus. Practically in some recipients such as those with cystic fibrosis, it is often necessary to remove bulky lymph nodes to facilitate the exposure and dissection of the vessels. Nevertheless, excessive lymphadenectomy should be avoided to prevent hardly controllable bleeding in the posterior mediastinum or some catastrophic complications

Video 4. Pre... ...of the hilum

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Figure 9 Preparation of the hilum. The bronchial stump is shortened until the main bronchus is reached. Careful haemostasis of the bronchial arteries must be performed, as they will be on the posterior part of the lung when the implantation is over. The arterial stump is divided as far as possible below the vena cava. Pericardial fluid can be seen when the pericardium is opened. After the dissection, a clamp is put on the main artery to check the safety of the anastomosis. Similarly, the veins are divided as far as possible. A suture of prolene is used to pull the phrenic nerve upward, and avoid traction and trauma during the implantation (11).

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Figure 10 Bronchial anastomosis. The first stitch between the two bronchial stomps is placed at the upper corner.

such as esophageal perforation.

After the vascular clamps have been chosen, they are removed for the bronchial anastomosis, the first one performed. Prolene traction sutures are placed on the pericardium, and between the PA and the pericardium for a better exposure of the bronchus (*Figure 9*).

Pulmonary implantation

Cold gauze sponges are placed in the posterior aspect of the pleural cavity, before the graft is positioned there. We perform the bronchial anastomosis first, then the arterial one, and finally the atrial one. Some teams choose a different sequence, starting with the venous anastomosis, as it is the deepest, followed by the PA and finally the bronchus.

Bronchial anastomosis (*Figure 10*)

Great care must be taken while performing this anastomosis, as complications, especially ischemic ones, are associated with higher morbidity and mortality rates (12). A flap, either from the recipient surrounding fatty tissues, an inter-costal muscle, or using the donor pericardium, reinforces the anastomosis. The right bronchus is anatomically shorter and wider than the left one, but there are otherwise no differences between the two anastomoses.

Different techniques can be used to perform the bronchial anastomosis (13-16):

- ❖ One continuous running suture on the whole circumference of the anastomosis;
- ❖ A posterior running suture on the membranous part of the bronchus, and separate stitches on the cartilaginous part;
- ❖ A telescopic suture when the diameters of the two bronchi are too different and major discrepancy is expected. This technique carries a higher rate of anastomotic complications.

We usually perform an end-to-end technique with a double needle slowly absorbable monofilament (PDS 3.0), starting the running suture at the upper part of the membranous wall of the bronchi consisting of the posterior aspect of the anastomosis. We use the same suture for the anterior cartilaginous aspect of the anastomosis and tie the two ends of the stitch in the middle. The chosen flap is then placed around the suture to prevent arterio-bronchial fistula (*Figure 11*).

Re-anastomosis of the bronchial arteries has been described to increase vascularization of the sutured bronchus, but it is a long and complex procedure, without strong evidence of its benefit to prevent later complications (18,19). It is mostly not used anymore.

Arterial anastomosis (*Figure 12*)

The arterial anastomosis is the second one we perform. A vascular clamp must be placed as far as possible on the proximal part of the PA, while the surgeon gently pulls the arterial stump towards him. It is then blocked with a loop fixed to the operative fields, to diminish the heart-induced

Video 5. Bronchial anastomosis

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Figure 11 Bronchial anastomosis. A PDS 3.0 is placed at the upper corners of the two bronchial stumps. The length of each side must be carefully assessed. The membranous part of the anastomosis is sutured with a running suture. Each side of the suture is used to perform a running stitch one from the upper corner towards the middle of the anterior side of the anastomosis, and one from the lower part. The congruence of the anastomosis must be checked, a flap can be placed on the anastomosis (pericardial tissue, fat tissue, …) (17).
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Figure 12 Arterial anastomosis.

movements.

The two arterial stumps are cut to achieve equivalent diameters on both sides. The surgeon must be careful to avoid any excessive length of the artery, which can lead to later obstructive plication of the anastomosis.

We perform an end-to-end suture with a non-absorbable suture of 5.0, run in a similar fashion as the bronchial anastomosis. The posterior suture is performed first with a double-needle suture, from the top to the bottom. Both sides of the suture are then used to perform running stitches for the anterior wall. The two halves of the suture are left on a clamp, and they will be tied together after the flush has been done (*Figure 13*).

Atrial anastomosis (*Figure 14*)

The atrial anastomosis is the last one we perform. A clamp

Video 6. Arterial anastomosis

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Figure 13 Arterial anastomosis. The length of the arterial stump is checked. The running suture starts at the upper end of the anastomosis with a non-absorbable suture of 5.0. Care should be taken to avoid transluminal stitches. Once the anterior wall is finished, both sides of the stitch are placed on a clamp and will be tied after the flush (20).
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Figure 14 Atrial anastomosis.

with a 120° angle is placed as far as possible in the pericardial space, while taking care not to injure a coronary artery, or alter the hemodynamic function. The clamp, as for the PA, can be stabilized with a stitch fixed on the operative field.

The stumps of the veins are pulled with atraumatic clamps, and are then opened by resection of the stapler lines. The two openings are joined after connection has been made with a dissector, creating one unique opening of the left atrium and allowing the surgeon to perform a single anastomosis.

We use the same technique as for the PA with an end-to-end anastomosis, performed by a running suture with a single non absorbable, double needled 4.0 stitch. Then, both surgical and the anesthesiological teams prepare for lung reperfusion (*Figure 15*).

Perfusion and ventilation in the new lung

For the transplanted lung to be functional, three points

Figure 15 Atrial anastomosis. The two extremities of the recipient veins are opened, a dissector is placed between them and the opening is completed. With a technique similar to the one used for the artery the anastomosis is performed with a running stitch of non resorbable 4.0 suture (21).
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Figure 16 Retrograde flush. A small opening of the running stitch on the anterior part of the arterial anastomosis is created while the atrial clamp is gently opened. Once the vascular structures are filled, the arterial suture is tied (22).
Available online: http://www.asvide.com/article/view/24501

must be checked:

❖ De-airing the vascular bed of the lung to avoid cerebral or coronary embolisms;
❖ Performing a progressive artery declamping in order to achieve a low pressure lung reperfusion;
❖ Performing a protective lung reventilation: FiO_2 of 40%, a tidal volume of 5 to 7 mL/kg of donor body weight, and a PEEP of 5 cmH_2O.

A retrograde and then anterograde flush is performed. Removing the atrial clamp insures a low pressure lung reperfusion. Progressive PA declamping also aims to prevent

pulmonary graft dysfunction resulting from reperfusion edema.

Retrograde flush

The flush is performed from the atrial anastomosis, with a low pressure (15 mmHg), towards the PA. The atrial clamp is slowly opened while the arterial one is left in place to prevent massive hemorrhage in case of a misfit anastomosis. Gentle ventilation helps the flush: volumes of 5 mL/kg, positive expiratory pressure of 5 cmH_2O, FiO_2 of 40%. When the vascular structures are filled, the retrograde de-airing is performed through the arterial suture, by opening the anterior wall of the anastomosis (*Figure 16*).

The atrial clamp is then closed again and an anterograde flush by opening the arterial clamp is performed. The arterial suture is tied while the flush continues at the atrial anastomosis. After a few minutes the atrial suture is tied as well. The lung is functioning again, with as protective a ventilation as possible (low FiO_2 and volume ventilation).

Anterograde flush

The arterial clamp is opened first while the atrial clamp is kept closed. This flush is performed with a high pressure (except under ECMO), meaning that the arterial clamp should be opened slowly and carefully. The flush is performed first at the arterial suture, the vascular structures are filling up, and the atrial anastomosis is finally flushed. Gentle ventilation of the lung is given as previously described.

The arterial clamp is closed, and a retrograde flush is performed by opening the atrial clamp. The suture is tied, the atrial clamp is left opened, and the arterial clamp is slowly opened.

In case of CBP, the pump flow must be decreased during the flush to allow a minimal pressure in the PA.

The lung is also rewarmed using hot saline solution to wash the pleural space. The surgical and anesthesiological teams wait for approximately 20 minutes to assess the function of the new lung (hemodynamic status, blood gas) before the other side is started. While waiting, the surgeon checks for any potential sources of bleeding, haemostatic patches can be placed on the anastomoses, and chest tubes (one apical, and one posterobasal) are placed in the chest cavity. The chest wall is not closed until the other side is finished.

The same technique is used for the second side. Due to anatomical features however, hemodynamic instability may occur during the operation on the left side, especially because of the necessity of retracting the heart to facilitate exposure of the left hilum in the rather small chest cavities

of some recipients. In this situation, when the operative approach consists of a bilateral anterolateral thoracotomy, the sternum should be divided transversally and the pericardium opened anteriorly to expose the heart. The heart is then lifted out of the pericardial sac and maintained gently in that position. This maneuver generally improves hemodynamics while exposing the left hilum adequately for graft implantation. Nevertheless, intraoperative ECMO may become necessary due to insufficient oxygenation on single-lung ventilation, hemodynamic instability, or the onset of reperfusion edema in the first implanted lung, especially in patients with secondary pulmonary arterial hypertension. Indeed, clamping of the artery is the crucial point of the second half of the transplant, as the newly implanted lung will receive full cardiac output and will have to provide the entire gas exchange. This is often the point where cardiopulmonary support is needed, if it has not been used before.

Usual criteria for ECMO, whether during the first lung implantation or the second one, are:

❖ Decrease of the cardiac index <1.5 L/min;
❖ Median pulmonary arterial pressure over 40–50 mmHg despite optimal nitric oxide therapy;
❖ SaO_2 <90% or major respiratory acidosis with a FiO_2 of 100% and optimal ventilation;
❖ Dysfunction of the right ventricle: hypokinesis, dilatation, paradoxical septum on echocardiography.

Thoracotomy closure

At the end of the procedure, after placement of at least two chest tubes in each pleural cavity, both thoracotomies are closed in a similar way as usual thoracic procedures. If the sternum has been opened, it is closed with separated stitches of steel suture. We also usually use an X steel point including the sternum and the intercostal space or only the intercostal space in its mediastinal part to prevent sternal pseudoarthrosis.

At this point, the patient can become hemodynamically instable, the chest wall causing cardiac tamponade, especially when reperfusion edema has occurred. It may be necessary for the surgeon to perform lung volume reduction to allow definitive closure. Wedge resections "on demand" are usually performed. However, on the right side, a middle lobectomy can be safely done, while on the left side resection of the lingula may be performed as well. If volume reduction is not sufficient, the chest wall can be left opened with a delayed closure until the edema has resolved.

The anesthesiologist changes the double-lumen tube for a conventional single-lumen tube before the patient is transferred to the intensive care unit.

The indications and management of post-operative ECMO are addressed elsewhere.

Technical problems and management

Vascular stenosis

Stenosis is the main complication of vascular sutures. Five mechanisms can be involved and should addressed:

❖ Transluminal stitch through both walls of the suture;
❖ Excessive tying of the running suture performed on a low pressure vessel;
❖ Inadequate positioning of the two stumps leading to a torsion of the anastomosis;
❖ Excessive length of vessel and a subsequent obstructive plication;
❖ External compression of the vessel by excess of the peribronchial tissue wrapping the bronchial anastomosis.

Vascular injury

The veins or the artery can also be injured during the procurement.

For the artery, if the wound is proximal, it will be removed while preparing the stump and will have no consequences, provided that a sufficient length of vessel is available at the level of the recipient artery. If the wound is distal, direct suture with non-absorbable 5.0 suture should be performed, and additional patch repair using remaining donor vessels can also be useful.

For the veins, when the inferior vein has been injured while dividing the pulmonary ligament, careful reconstruction by direct suture can be performed.

If the injury is more important, for examples when the ostia are involved or if the cuff is too short, reconstruction with pericardial tissue can be performed (23-25) (*Figure 17*).

Anatomical variations

The upper right lobe bronchus in the donor lung can originate directly from the trachea. If it is a segmentary bronchus, it can be sacrificed and the collateral airway structures will be enough for ventilation of the upper
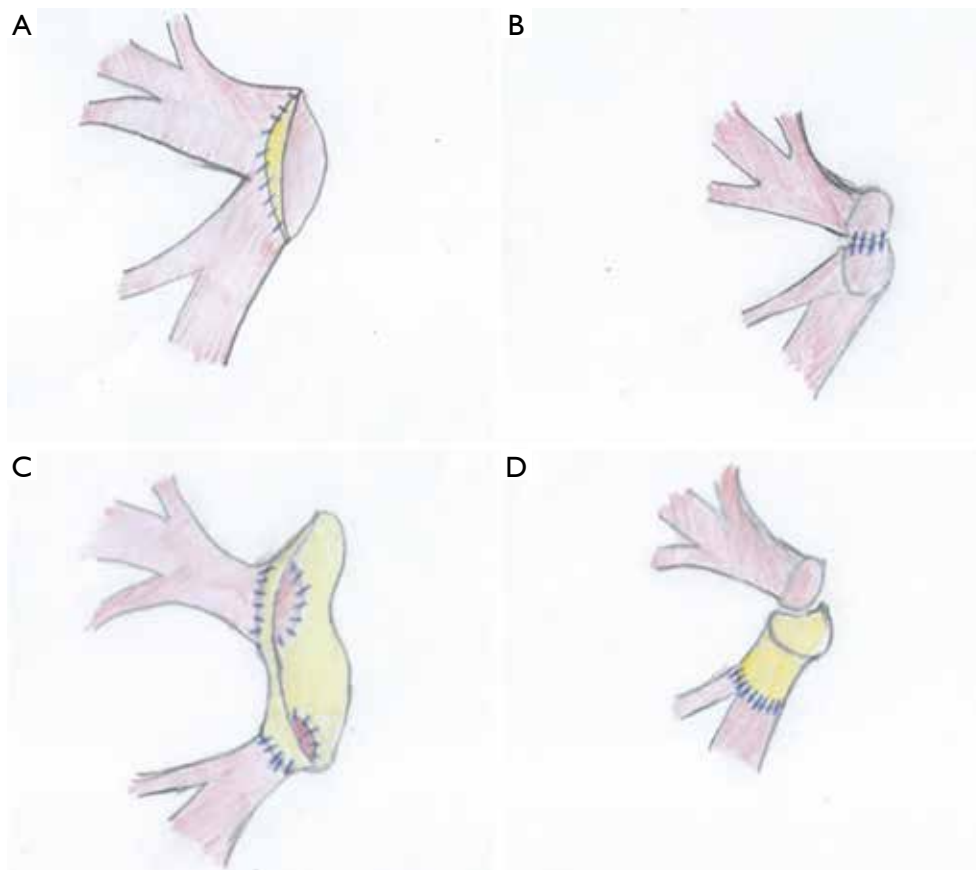
Figure 17 Techniques of atrial reparation. (A) The atrial cuff is too short. A pericardial patch is placed partially or totally on the circumference of the cuff; (B) the veins have been split accidentally. A running suture is performed for reconstruction of the cuff; (C) the veins have been split with an associated defect of length. Both veins are sutured to a cuff of pericardium; (D) one vein has been shortened. It is reconstructed with a pericardial bridge.

lobe. If the whole upper right lobe bronchus is involved a lobectomy can be performed, or the bronchus can be sutured to the truncus intermedius (26). Another alternative would be to perform left lung transplantation only.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary lung fibrosis. N Engl J Med 1986;314:1140-5.
2. Patterson GA, Cooper JD, Goldman B, et al. Technique of successful clinical double-lung transplantation. Ann Thorac Surg 1988;45:626-33.
3. Patterson GA, Todd TR, Cooper JD, et al. Airway complications after double lung transplantation. J Thorac Cardiovasc Surg 1990;99:14-20.
4. Bisson A, Bonnette P. A new technique for double lung transplantation. "Bilateral single lung transplantation". J Thorac Cardiovasc Surg 1992;103:40-6.
5. Pasque MK, Cooper JD, Kaiser LR, et al. Improved technique for bilateral lung transplantation:rationale and clinical experience. Ann Thorac Surg 1990;49:785-91.
6. Kaiser LR, Pasque MK, Trulock EP, et al. Bilateral sequential lung transplantation:the procedure of choice for double lung replacement. Ann Thorac Surg 1991;52:438-45.

7. Fischer S, Strüber M, Simon AR, et al. Video-assisted minimally invasive approach in clinical bilateral lung transplantation. J Thorac Cardiovasc Surg 2001;122:1196-98.

8. Gust L, D'Journo XB, Brioude G, et al. Lung dissection. Asvide 2018;5:432. Available online: http://www.asvide.com/article/view/24494

9. Gust L, D'Journo XB, Brioude G, et al. Preparation of the graft. Asvide 2018;5:433. Available online: http://www.asvide.com/article/view/24495

10. Gust L, D'Journo XB, Brioude G, et al. Explantation of the native lung. Asvide 2018;5:434. Available online: http://www.asvide.com/article/view/24496

11. Gust L, D'Journo XB, Brioude G, et al. Preparation of the hilum. Asvide 2018;5:435. Available online: http://www.asvide.com/article/view/24497

12. Van De Wauwer C, Van Raemdonck D, Verleden GM, et al. Risk factors for airway complications within the first year after lung transplantation Eur J Cardiothorac Surg 2007;31:703-10.

13. Parekh K, Patterson A. Technical considerations in adult lung transplantation. Semin Thorac Cardiovasc Surg 2004;16:322-32.

14. Aigner C, Jaksch P, Seebacher G, et al. Single running suture--the new standard technique for bronchial anastomoses in lung transplantation. Eur J Cardiothorac Surg 2003;23:488-93.

15. Garfein ES, Ginsberg ME, Gorenstein L, et al. Superiority of end-to-end versus telescoped bronchial anastomosis in single lung transplantation for pulmonary emphysema. J Thorac Cardiovasc Surg 2001;121:149-154

16. Garfein ES, McGregor CC, Galantowicz ME, et al. Deleterious effects of telescoped bronchial anastomosis in single and bilateral lung transplantation. Ann Transplant 2000;5:5-11.

17. Gust L, D'Journo XB, Brioude G, et al. Bronchial anastomosis. Asvide 2018;5:436. Available online: http://www.asvide.com/article/view/24498

18. Daly RC, McGregor CGA. Routine immediate direct bronchial artery revascularisation for single lung transplantation. Ann Thorac Surg 1994;57:1446-52.

19. Yacoub M, Al-Kattan KM, Tadjkarimi S, et al. Medium term results of direct bronchial artery revascularisation using IMA for single lung transplantation (SLT with direct revascularisation). Eur J Cardiothorac Surg 1997;11:1030-6.

20. Gust L, D'Journo XB, Brioude G, et al. Arterial anastomosis. Asvide 2018;5:437. Available online: http://www.asvide.com/article/view/24499

21. Gust L, D'Journo XB, Brioude G, et al. Atrial anastomosis. Asvide 2018;5:438. Available online: http://www.asvide.com/article/view/24500

22. Gust L, D'Journo XB, Brioude G, et al. Retrograde flush. Asvide 2018;5:439. Available online: http://www.asvide.com/article/view/24501

23. Casula RP, Stoica SC, Wallwork J, et al. Pulmonary vein augmentation for single lung transplantation. Ann Thorac Surg 2001;71:1373-4.

24. Oto T, Rabinov M, Negri J, et al. Techniques of reconstruction for inadequate donor left atrial cuff in lung transplantation. Ann Thorac Surg 2006;81:1199-204.

25. D'Journo XB, Gariboldi V, Trousse D, et al. Techniques de transplantation bipulmonaires. EMC - Techniques chirurgicales - Thorax 2011:1-15 [Article 42-440-C].

26. Schmidt F, McGiffin DC, Zorn G, et al. Management of congenital abnormalities of the donor lung. Ann Thorac Surg 2001;72:935-7.

Heart-lung transplantation: current indications, prognosis and specific considerations

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Abstract: Heart-lung transplantation (HLTx) is currently the best treatment for patients who have end-stage heart and lung failure. Idiopathic pulmonary arterial hypertension (IPAH) was the main indication for HLTx in the 1980s. However, when studies showed resolution of right ventricular dysfunction after double-lung transplantation (DLTx), this last procedure became the preferred option for end-stage IPAH. Currently, the main indication of HLTx is congenital heart disease (CHD), followed by acquired heart disease combined with pulmonary hypertension and/or intrinsic lung disease. Although early posttransplant survival remains lower after HLTx than after lung transplantation, careful patient selection combined with surgical advances are producing improvements. Here, we review the practice patterns, trends, and outcomes of HLTx worldwide.

Keywords: Heart-lung transplantation (HLTx); prognosis; indication; survival

Introduction

Over 25 years of experimentation were required to allow the first successful heart-lung transplantation (HLTx) in a human patient (1,2). In the late 1960s and early 1970s, HLTx was performed in 3 patients, all of whom died rapidly, the longest survival being 23 days. Then, in the early 1980s, a group in Stanford performed HLTx in 3 patients with pulmonary vascular disease, of whom 2 achieved long-term survivals (3). The number of HLTx procedures recorded in the registry maintained by the International Society of Heart and Lung Transplantation (ISHLT) peaked in 1989 then declined to less than 100 per year at present (4). This change is partly ascribable to the predominant initial use of HLTx in patients with pulmonary vascular disease or

cystic fibrosis (CF), who are now generally managed by lung transplantation. The indications for HLTx continue to evolve. Currently, HLTx is considered the best option in selected patients who have end-stage heart and lung failure.

Indications

Complex congenital heart disease (CHD) complicated by Eisenmenger syndrome is the most common indication of HLTx (*Table 1*). HLTx is also indicated in patients who have both end-stage lung disease and either refractory left ventricular failure or objectively documented fibrosis or infarction of the right ventricle with right ventricular failure (6). The ISHLT registry has a total of 3,879 HLTxs performed in adults between January 1982 and June

Table 1 Diagnoses in adults managed with heart-lung transplantation between January 1982 and June 2016

Diagnosis	Number (%)
PH-not IPAH	1,204 (37.5)
IPAH	953 (29.7)
CF	462 (14.4)
COPD	141 (4.4)
IIP	113 (3.5)
A1ATD	63 (2.0)
Sarcoidosis	58 (1.8)
ILD-not IIP	47 (1.5)
Retransplant	42 (1.3)
Non-CF bronchiectasis	33 (1.0)
BOS	23 (0.7)
Other	74 (2.3)

From Lund *et al.* (5), with permission. PH, pulmonary hypertension; IPAH, idiopathic pulmonary arterial hypertension; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; IIP, idiopathic interstitial pneumonia; A1ATD, alpha-1 antitrypsin deficiency; ILD, interstitial lung disease; BOS, bronchiolitis obliterative syndrome.

2015 (4). The highest annual number of HLTxs in adults was 226, in 1989. The subsequent decline reflects advances in other treatments for pulmonary hypertension and heart failure combined with the use of isolated heart or lung transplantation in patients who would previously have been managed by HLTx. In the past decade, the annual number of HLTxs was only 49 to 92 worldwide (4). According to the ISHLT registry, the most common indication for HLTx from 1982 to 1991 was PAH, followed by CHD and CF (7). Nearly all patients who have PAH or CF are now managed with double-lung transplantation (DLTx) alone. Nevertheless, PAH accounted for as many as 27.3% of HLTx procedures performed between January 2004 and June 2015, compared to 35.5% for CHD (35%) and 11% for cardiomyopathy (11%) (4).

Candidates

Conditions for which HLTx is now viewed as the best transplant option include Eisenmenger syndrome complicating complex CHD, failed CHD repair, uncorrectable CHD, and severe left ventricular failure.

As cardiomyopathy can result in right ventricular failure and early death, heart transplantation (HTx) may not be a good option when the right heart catheterization shows pulmonary vascular resistance (PVR) values above >3 Wood units or a transpulmonary pressure gradient above 15 mmHg (8). However, isolated HTx is usually considered an acceptable option in patients whose PVR falls below 4 Wood units (320 dyn·s/cm^5) with treatment. In contrast, HLTx may be an alternative to HTx in patients whose PVR remains high despite treatment.

Uncertainty long prevailed regarding the best transplantation option in patients with PAH. However, in several studies, patients with PAH had similar outcomes after DLTx as after HLTx, even when they had right ventricular failure (9,10). After DLTx, PVR may return to normal, and acute or chronic right ventricular failure complicating severe pulmonary hypertension may resolve. As long as the left ventricle is normal, HLTx is not required, and DLTx should be considered the procedure of choice in virtually all patients with PAH (11). A 2015 study of patients in the Scientific Registry of Transplant Recipients who had right ventricular failure complicating idiopathic PAH demonstrated similar long-term survival between HLTx and DLTx recipients (10).

However, transplant centers vary widely regarding the degree of right and left ventricular function impairment deemed consistent with DLTx. Cutoffs below which DLTx is deemed inappropriate vary between 10% and 25% for the right ventricular ejection fraction and between 32% and 50% for the left ventricular ejection fraction (LVEF) (12-14). According to one group, an LVEF of 30% to 35% may be compatible with DLTx provided the cardiac index is above 2.2 L/min/m² and the pulmonary capillary wedge pressure or left ventricular end-diastolic pressure is no greater than 15 mmHg, as assessed by right heart catheterization (11).

Patients with end-stage lung disease and reparable heart abnormalities may be eligible for DLTx combined with the appropriate cardiac procedure (e.g., reparative CHD surgery, coronary artery bypass grafting, or surgery to repair or replace a valve) (15). Waiting times are shorter and the likelihood of receiving a transplant higher with DLTx than with HLTx. Transplant allocation practices in North America and Europe have made it extremely difficult to obtain heart-lung blocks (16,17).

Extracorporeal membrane oxygenation (ECMO) as a bridge to DLTx is now a recognized treatment option in patients with end-stage lung disease. However, data on ECMO in patients awaiting transplants are limited, and the

potential benefits of ECMO as a bridge to HLTx in adults remain unclear (18,19). Data from small observational cohorts suggest that ECMO bridging is safe and provides good short-term outcomes (11). However, data on HLTx recipients recorded in the United Network for Organ Sharing (UNOS) database between 1995 and 2011 show poorer survival in patients who required pretransplant invasive mechanical ventilation or ECMO than in similar controls: 1-month survival was 20% in the ECMO group and 83.5% in the control group, and corresponding values for 5-year survival were 20% and 45.4% (20).

Increased pretransplant mortality risk among HLTx candidates

Among HLTx candidates in France in 2011–2016, within 12 months after listing, only 33% received transplants and as many as 32% were taken off the list because of death or severe clinical deterioration (17). Factors explaining the decreased use of HLTx may include diminished availability due to allocation practices, HLA antibody development due to blood transfusions, and shorter height of patients with CHD (21). Most HLTx candidates have a history of cardiovascular surgery, which results in the development of adhesions, anatomical alterations, and collateral blood vessels, thereby creating technical difficulties (22). HLTx requires cardiopulmonary bypass, which carries a risk of complications including coagulation disorders, decreased brain perfusion, acute kidney injury, and arrhythmias (22). Consequently, either HTX or DLTx deserves preference if allowed by the patient's condition.

Time of listing

According to general recommendations, transplantation should be considered when optimal medical therapy is expected to provide no more than 2 years' survival and there are no contraindications (6). However, debate continues to surround the best time for HLTx. Indeed, in patients with Eisenmenger syndrome, in whom HLTx is the preferred procedure, long-term survival is unpredictable and patient-specific. In addition, referral for HLTx is in order in patients with worsening ventricular failure and pulmonary hypertension. Factors that govern listing decisions include cardiac anatomy, hemodynamic status, and the general condition of the patient. Another criterion for transplant candidacy evaluation is a decline in quality of life related to worsening cardiopulmonary failure with increasingly

frequent hospital admissions. Listing criteria include New York Heart Association (NYHA) class III or IV right ventricular failure in a patient receiving optimal medical treatment, with a cardiac index below 2 L/min/m^2 and right atrial pressure above 15 mmHg (6). Conditions that may require earlier listing include documented or possible pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis with significant hemoptysis (6).

Postoperative management and complications

Postoperative care is the same after HLTx as after DLTx or single-lung transplantation (SLTx). Indeed, the lungs, and not the heart, are at the origin of most postoperative complications, including infections and acute and chronic rejection (21).

The induced immunosuppression after HLTx is comparable to that after DLTx or SLTx, i.e., usually more profound than after HTx. The follow-up of HLTx recipients includes closely-spaced lung function tests, chest radiographs, and outpatient assessments. The optimal interval between surveillance bronchoscopies after HLTx is not agreed on, and most centers usually apply their protocol designed for lung transplant recipients. Protocols for aspirin, beta-blocker, and statin therapy in HLTx recipients vary across centers, reflecting the absence of clear guidelines.

Acute cellular rejection in either the heart or lungs after HLTx is less common than after isolated heart or lung transplantation (23). Of note, acute cellular rejection involves the lungs more often than the heart (23). Acute cellular rejection of the heart may be synchronous or asynchronous with lung rejection but is uncommon and usually occurs early (23). Surveillance endomyocardial biopsies, although obtained routinely after HTx to look for asymptomatic rejection, is rarely performed after HLTx, particularly beyond 4 to 6 months (24). In addition, there is some recent evidence that using echocardiography alone to monitor heart allograft function is associated with similar outcomes to those seen with routine endomyocardial biopsies (25).

Most infectious complications after HLTx involve the lungs and are comparable in frequency to those recorded after DLTx or SLTx. In HLTx recipients, the curative and prophylactic treatment of infectious complications is no different than after lung transplantation.

Long-term outcomes are comparable after HLTx and after DLTx/SLTx, i.e., significantly worse than after
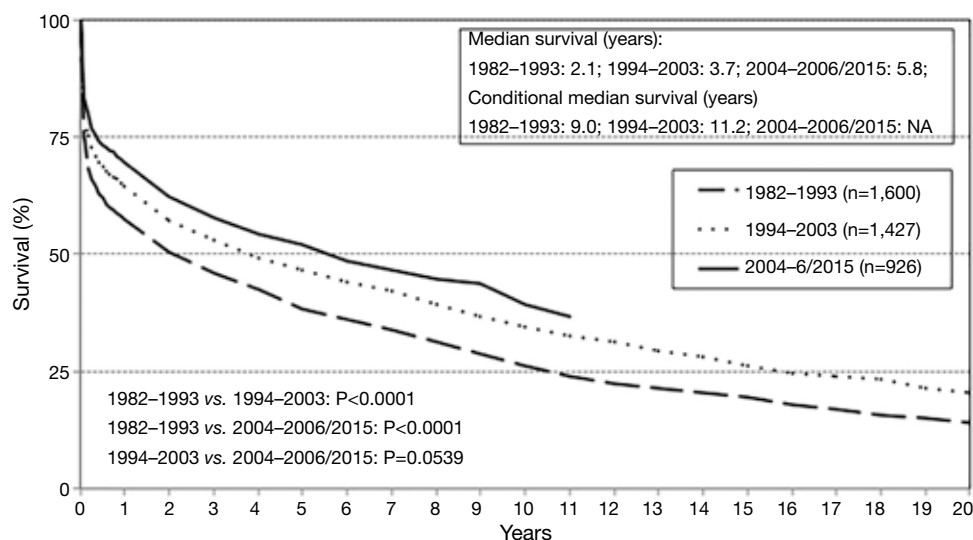
Figure 1 Kaplan-Meier survival curve for adult HLTx recipients transplanted in 1982–1993, 1994–2003, and 2004–2015. Conditional median survival is defined as the time to 50% survival in 1-year survivors [from Lund *et al.* (5), with permission]. HLTx, heart-lung transplantation.

HTx. Most complications in HLTx recipients involve the lungs (21). HLTx may be followed by chronic rejection of the heart, the lungs, or both. Coronary artery vasculopathy (CAV) occurs less often than bronchiolitis obliterans syndrome (BOS) after HLTx. Thus, in one study, rates of CAV 1, 3, 5, and 10 years after HLTx were 3%, 7%, 9%, and 27%, respectively, compared to 8%, 27%, 42%, and 62% for BOS (26).

Survival

Since the first HLTx procedures in the 1980s, survival has improved steadily. The outcomes of 23 HLTx procedures performed in 22 patients during the first 3.5 years of the Stanford program were reported in 1985 (27). The diagnoses were Eisenmenger syndrome with CHD in 12 patients and idiopathic PAH in 10 patients. Of the 22 patients, 6 (27%) died within 30 days or before hospital discharge. Predicted actuarial survival was 71% after 1 year and 57% after 2 years (27). The ISHLT registry has data for 1,216 patients transplanted in 1982–1991, whose outcomes were similar, with 25.4% early mortality and survival rates at 1, 2, 5, and 10 years of 56%, 49%, 37.7%, and 26%, respectively (28). In contrast, the 2016 ISHLT report for 2004–2014 indicates far higher survival rates of 63%, 52%, 45%, and 32% after 1, 2, 5, and 10 years, respectively, and a median survival of 5.8 years (26) (*Figure 1*). Although long-

term survival has improved, the most substantial gains have been achieved during the early posttransplant period. In recipients transplanted in 2004–2014 who were alive after 1 year, median survival was longer than 10 years (26). Factors that explain these survival gains include improved selection of patients to HLTx, advances in surgical technique, the development of better immunosuppressive regimens for preventing rejection, and new insights into the factors associated with morbidity and mortality.

Causes and predictors of mortality

Survival after HLTx has improved over time, although the causes of death have remained unchanged. During the first month after HLTx, the main causes of death are posttransplant graft failure, technical complications, and infection, whereas BOS and chronic lung allograft dysfunction cause the most deaths beyond the first year. Mechanical ventilation or circulatory support before HLTx is associated with poorer outcomes. Thus, an analysis of the UNOS database for HLTx procedures done in 1995–2011 showed lower survival among patients who required pre-transplant ventilation or ECMO as in controls similar for recipient and donor age and gender and for ischemic time, both during the first month (20% with *vs.* 83.5% without ECMO) and after 5 years (20% *vs.* 45.4%, P<0.0001) (20). These findings indicate a marked difference in outcomes

depending on pre-HLTx severity. Due to the small number of HLTx procedures performed each year, studies of risk factors for death are scarce and produced limited findings. A 2016 study of ISHLT data found associations linking higher mortality to older donor age and to HLTx for conditions other than idiopathic PAH. Patients managed at centers with low HLTx volumes did not have higher mortality, but volume was low in all centers (28).

Long-term immunosuppressive therapy is the most common source of morbidity after HLTx. Among HLTx recipients entered in the ISHLT registry in 1994–2013, 88.1% had hypertension and 70% hyperlipidemia 5 years after transplantation. Furthermore, 45.5% had kidney dysfunction, which required dialysis in 2.1% and kidney transplantation in 1.1%. Consistent with the statement above that early complications usually involve the lungs, BOS occurred in 28.7% of patients, compared to 8.2% for CAV. Retransplantation was uncommon, with only 57 HLTx recipients undergoing a second HLTx from January 1982 through June 2015 (26). The finding of poorer outcomes after repeat HLTx than after primary HLTx warrants caution when considering a second HLTx procedure.

Benefits of HLTx depending on the indication

Pulmonary hypertension with right heart failure

Outcomes of SLTx, DLTx, and HLTx done in 1989–1993 in 30 patients with Eisenmenger syndrome and 27 with primary pulmonary hypertension were assessed retrospectively (12). Indications for HLTx were LVEF below 35%, significant coronary artery disease, and Eisenmenger syndrome complicating complex CHD. Mortality after 1 to 3 months was similar in these three groups. However, the SLTx recipients had lower 1-year survival (38%) and a significantly higher incidence of graft failure (SLTx, 82%; DLTx, 59%; and HLTx, 33%; P<0.05). In a retrospective comparison of outcomes of HLTx (n=157) and DLTx (n=67) performed in 1986–2008, our group found no differences in 1-, 5-, 10-, or 15-year survival (HLTx: 70%, 50%, 39%, and 26% vs. DLTx, 79%, 52%, 43%, and 30%, respectively; P=0.46), despite more severe pretransplant disease in the HLTx group (with greater severity of right ventricular, liver, and kidney dysfunction and higher inotropic support requirements; P<0.05 for each) (9). The proportion of patients free of BOS after 10 years was significantly higher after HLTx than after DLTx (79% vs. 74%, P=0.035). In a Scientific Registry of Transplant Recipients study of HLTx and DLTx procedures performed

in 1987–2012, the comparison of 261 HLTx patients and 667 DLTx patients showed no difference in overall survival (10). However, patients who required ICU admission before transplantation had a significantly better 8-year survival rate after HLTx than after DLTx (40% vs. 20%, P=0.043). Thus, patients with severe right heart failure may fare better overall with HLTx than with lung transplantation alone.

CHD

About 75% to 85% of patients with CHD survive to adulthood, and about 10% may then eventually require transplantation at some point (29). Decisions about whether and when to transplant incorporate many factors, including the type of defect, severity of the disease, and availability of organs.

Recent studies have identified markers of poor outcome in large patient cohorts with CHD (30,31). First, in selected patients with reparable CHD, same-stage LTx may constitute an alternative to HLTx. A retrospective study compared outcomes after DLTx with prior or same-stage CHD repair (n=35) and after HLTx (n=16) performed in pediatric patients in 1990–2003 (15). The most common diagnoses in the DLTx and repair group were ventricular septal defect (VSD), pulmonary venous obstruction, and pulmonary atresia. The two groups were similar for 1-, 3-, and 5-year survival (HLTx: 66.5%, 66.5%, and 60%; and DLTx, 62.9%, 51.4%, and 51.4%; respectively; P=0.852) and for survival without BOS (HLTx: 77.8%, 51.9%, and 38.9%; and DLTx: 72.9%, 54.7%, and 54.7%; respectively; P=0.442). In this population, HLTx was the preferred option in patients with multiple and complex CHD, and DLTx was considered only when the expected ischemic time needed for CHD repair was no greater than 60 min. Other studies demonstrated better outcomes after HLTx than after DLTx or SLTx with or without CHD repair in patients with Eisenmenger syndrome. A study of UNOS and ISHLT registry data compared long-term outcomes of HLTx (n=430), DLTx (n=106), and SLTx (n=69) in patients with CHD and Eisenmenger syndrome (32). By multivariable analysis, survival was significantly better with HLTx than with lung transplantation in patients with VSD (HLTx for VSD vs. atrial septal defect or patent ductus arteriosus: risk ratio, 0.517; P=0.0001; lung transplantation vs. HLTx for VSD, risk ratio, 1.817; P=0.035), and HLTx provided a significantly higher 1-year survival rate in the groups with VSD (71.4%) and multiple congenital anomalies (77.6%, P=0.011) (33). Other studies found no

significant differences in HLTx outcomes in patients with *vs.* without Eisenmenger syndrome and suggested that HLTx was effective and safe in both groups (29,32,34-37).

Conclusions

Although in numerical decline for several decades, HLTx is currently viewed as the best procedure in carefully selected patients. Indeed, HLTx offers better survival to patients who have severe heart failure or complex CHD complicated with pulmonary hypertension. Furthermore, HLTx is associated with a usually lower ischemic time, producing better outcomes; a lower rate of BOS-related mortality; and significantly better postoperative cardiac function.

Compared to DLTx, HLTx does not produce worse outcomes when used to treat CHD or idiopathic PAH. Nonetheless, HLTx should be reserved for those patients with no other therapeutic options. In patients with Eisenmenger syndrome, the optimal timing of HLTx varies widely across patients. However, listing patients before their disease becomes so severe as to preclude good posttransplant outcomes is crucial.

The management of these patients with complex needs requires a multidisciplinary model. Close monitoring for declining function is imperative to ensure that patients are considered for HLTx listing in a timely manner. In patients with PAH, DLTx is the preferred treatment when heart function remains acceptable, whereas HLTx may need to be considered in the event of severe right and/or left heart failure.

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Footnote

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References

1. Webb WR, Howard HS. Cardio-pulmonary transplantation. Surg Forum 1957;8:313-7.
2. Lower RR, Stofer RC, Hurley EJ, Shumway NE. Complete homograft replacement of the heart and both lungs. Surgery 1961;50:842-5.
3. Reitz BA, Wallwork JL, Hunt SA, et al. Heart-lung transplantation: successful therapy for patients with pulmonary vascular disease. N Engl J Med 1982;306:557-64.
4. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant 2015;34:1264-77.
5. Lund LH, Khush KK, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-fourth Adult Heart Transplantation Report-2017; Focus Theme: Allograft ischemic time. J Heart Lung Transplant 2017;36:1037-46.
6. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.
7. Yusen RD, Christie JD, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Adult Lung and Heart-Lung Transplant Report--2013; focus theme: age. J Heart Lung Transplant 2013;32:965-78.
8. Mehra MR, Canter CE, Hannan MM, et al. The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: A 10-year update. J Heart Lung Transplant 2016;35:1-23.
9. Fadel E, Mercier O, Mussot S, et al. Long-term outcome of double-lung and heart-lung transplantation for pulmonary hypertension: a comparative retrospective study of 219 patients. Eur J Cardiothorac Surg 2010;38:277-84.
10. Hill C, Maxwell B, Boulate D, et al. Heart-lung vs. double-lung transplantation for idiopathic pulmonary arterial hypertension. Clin Transplant 2015;29:1067-75.
11. Toyoda Y, Toyoda Y. Heart-lung transplantation: adult indications and outcomes. J Thorac Dis 2014;6:1138-42.
12. Bando K, Armitage JM, Paradis IL, et al. Indications for and results of single, bilateral, and heart-lung transplantation for pulmonary hypertension. J Thorac Cardiovasc Surg 1994;108:1056-65.
13. Olland A, Falcoz PE, Canuet M, et al. Should we perform bilateral-lung or heart–lung transplantation for patients with pulmonary hypertension? Interact Cardiovasc Thorac Surg 2013;17:166-70.
14. Pielsticker EJ, Martinez FJ, Rubenfire M. Lung and heart-lung transplant practice patterns in pulmonary hypertension centers. J Heart Lung Transplant 2001;20:1297-304.
15. Choong CK, Sweet SC, Guthrie TJ, et al. Repair

of congenital heart lesions combined with lung transplantation for the treatment of severe pulmonary hypertension: a 13-year experience. J Thorac Cardiovasc Surg 2005;129:661-9.

16. Colvin-Adams M, Valapour M, Hertz M, et al. Lung and heart allocation in the United States. Am J Transplant 2012;12:3213-34.

17. Agence de la biomédecine - Le rapport annuel médical et scientifique 2016. Available online: https://www.agence-biomedecine.fr/annexes/bilan2016/donnees/organes/04-coeur-poumon/synthese.htm

18. Strueber M, Hoeper MM, Fischer S, et al. Bridge to thoracic organ transplantation in patients with pulmonary arterial hypertension using a pumpless lung assist device. Am J Transplant 2009;9:853-7.

19. Gregoric ID, Chandra D, Myers TJ, et al. Extracorporeal membrane oxygenation as a bridge to emergency heart-lung transplantation in a patient with idiopathic pulmonary arterial hypertension. J Heart Lung Transplant 2008;27:466-8.

20. Jayarajan SN, Taghavi S, Komaroff E, et al. Impact of extracorporeal membrane oxygenation or mechanical ventilation as bridge to combined heart-lung transplantation on short-term and long-term survival. Transplantation 2014;97:111-5.

21. Idrees JJ, Pettersson GB. State of the Art of Combined Heart-Lung Transplantation for Advanced Cardiac and Pulmonary Dysfunction. Curr Cardiol Rep 2016;18:36.

22. Pasupneti S, Dhillon G, Reitz B, et al. Combined Heart Lung Transplantation: An Updated Review of the Current Literature. Transplantation 2017;101:2297-302.

23. Pinderski LJ, Kirklin JK, McGiffin D, et al. Multi-organ transplantation: is there a protective effect against acute and chronic rejection? J Heart Lung Transplant 2005;24:1828-33.

24. Glanville AR, Imoto E, Baldwin JC, et al. The role of right ventricular endomyocardial biopsy in the long-term management of heart-lung transplant recipients. J Heart Transplant 1987;6:357-61.

25. Technology CM. 829 - Non Invasive Monitoring of Acute Allograft Rejection in Heart Transplantation: Long-term Outcomes of the "No Biopsy Approach." Available online: https://cslide-us.ctimeetingtech.com/ishlt2018/attendee/eposter/poster/819

26. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.

27. Dawkins KD, Jamieson SW, Hunt SA, et al. Long-term results, hemodynamics, and complications after combined heart and lung transplantation. Circulation 1985;71:919-26.

28. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-first adult lung and heart-lung transplant report--2014; focus theme: retransplantation. J Heart Lung Transplant 2014;33:1009-24.

29. Hosseinpour AR, Cullen S, Tsang VT. Transplantation for adults with congenital heart disease. Eur J Cardiothorac Surg 2006;30:508-14.

30. Kempny A, Hjortshøj CS, Gu H, et al. Predictors of Death in Contemporary Adult Patients With Eisenmenger Syndrome: A Multicenter Study. Circulation 2017;135:1432-40.

31. Hjortshøj CMS, Kempny A, Jensen AS, et al. Past and current cause-specific mortality in Eisenmenger syndrome. Eur Heart J 2017;38:2060-7.

32. Stoica SC, McNeil KD, Perreas K, et al. Heart-lung transplantation for Eisenmenger syndrome: early and long-term results. Ann Thorac Surg 2001;72:1887-91.

33. Waddell TK, Bennett L, Kennedy R, et al. Heart-lung or lung transplantation for Eisenmenger syndrome. J Heart Lung Transplant 2002;21:731-7.

34. Stoica SC, Perreas K, Sharples LD, et al. Heart-lung transplantation for Eisenmenger's syndrome: operative risks and late outcomes of 51 consecutive cases from a single institution. J Heart Lung Transplant 2001;20:173-4.

35. Hopkins WE, Ochoa LL, Richardson GW, et al. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. J Heart Lung Transplant 1996;15:100-5.

36. Goerler H, Simon A, Gohrbandt B, et al. Heart-lung and lung transplantation in grown-up congenital heart disease: long-term single centre experience. Eur J Cardiothorac Surg 2007;32:926-31.

37. Vouhé PR, Tamisier D, Le Bidois J, et al. Pediatric cardiac transplantation for congenital heart defects: surgical considerations and results. Ann Thorac Surg 1993;56:1239-47.

Pediatric lung transplantation

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Abstract: Pediatric lung transplantation has been undertaken since the 1980s, and it is today considered an accepted therapy option in carefully selected children with end-stage pulmonary diseases, providing carefully selected children a net survival benefit and improved health-related quality of life. Nowadays, >100 pediatric lung transplants are done worldwide every year. Here, specific pediatric aspects of lung transplantation are reviewed such as the surgical challenge, effects of immunosuppression on the developing pediatric immune system, and typical infections of childhood, as it is vital to comprehend that children undergoing lung transplants present a real challenge as children are not 'just small adults'. Further, an update on the management of the pediatric lung transplant patient is provided in this review, and future challenges outlined. Indications for lung transplantation in children are different compared to adults, the most common being cystic fibrosis (CF). However, the primary diagnoses leading to pediatric lung transplantation vary considerably by age group. Furthermore, there are regional differences regarding the primary indication for lung transplantation in children. Overall, early referral, careful patient selection and appropriate timing of listing are crucial to achieve real survival benefit. Although allograft function is to be preserved, immunosuppressant-related side effects are common in children post-transplantation. Strategies need to be put into practice to reduce drug-related side effects through careful therapeutic drug monitoring and lowering of target levels of immunosuppression, to avoid acute-reversible and chronic-irreversible renal damage. Instead of a "one fits all approach", tailored immunosuppression and a personalized therapy is to be advocated, particularly in children. Further, infectious complications are a common in children of all ages, accounting for almost 50% of death in the first year post-transplantation. However, chronic lung allograft dysfunction (CLAD) remains the major obstacle for improved long-term survival.

Keywords: Children; cystic fibrosis (CF); lung transplantation; pediatrics

Lung transplants in children have been undertaken since the 1980s, and nowadays, pediatric lung transplantation is considered as an accepted therapy option in carefully selected children with end-stage parenchymal and vascular pulmonary diseases, providing the well-selected pediatric candidate a net survival benefit and improved health-related quality of life (1-4).

The 2016 International Society for Heart and Lung Transplantation (ISHLT) Thoracic Transplant Registry Report shows that over 100 pediatric lung transplants per annum were reported to the Registry worldwide (5).

Since set-up of the ISHLT Pediatric Thoracic Transplant Registry, more than 2,000 pediatric lung transplant procedures have been reported in total to date; however, less than 30 centres carry out such procedures in children, the majority of centers perform less than 5 procedures per year (5). In Europe, pediatric lung transplants are done in predominantly in adult centers, with variable input of pediatricians. Despite this, very good results were achieved in pediatric lung transplantation as reported by individual adult centers (6). On the other hand, published data indicated that not only transplant center volume, but also specific pediatric expertise effects outcome of pediatric lung transplantation (7). Further, a recent analysis of
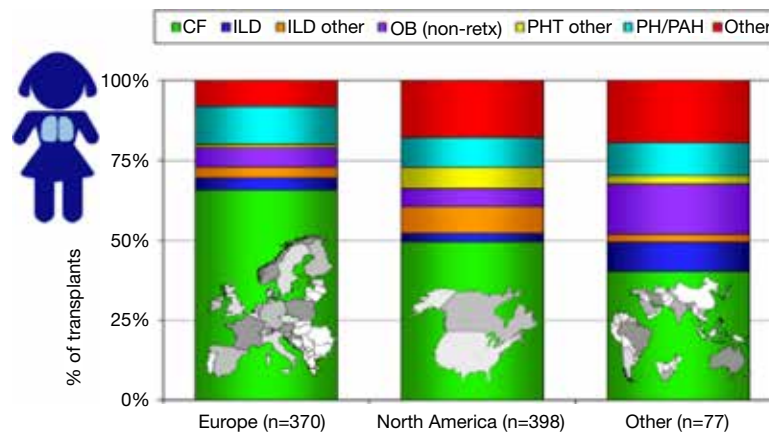
Figure 1 Underlying diagnoses in pediatric lung transplant recipients according to geographic region (lung transplant procedures performed between January 2008 and June 2015) (5) (with permission from the publisher). CF, cystic fibrosis; ILD, interstitial lung disease; OB, obliterative bronchiolitis; PHT, pulmonary hypertension; PH, pulmonary hypertension; PAH, pulmonary arterial hypertension.

United Network for Organ Sharing (UNOS) data analysis including more than 2,000 patients across 67 transplant centers revealed that particularly CF-specific expertise in cystic fibrosis (CF), the most common primary indication for pediatric lung transplantation, predicts better long-term outcome of lung transplantation for CF (7).

Nevertheless it is vital to comprehend that children undergoing lung transplantation present a challenge as children are not 'just small adults'. The surgical approach can be more challenging, and effects of immunosuppression in the developing immune system of a child, and psychosocial aspects, particularly in adolescents, have to be taken into consideration (2).

Primary indications for lung transplant, referral and transplant evaluation

Indications for lung transplants in children are different compared to adults. In adults, the most frequent primary indication for lung transplantation is chronic obstructive pulmonary disease (COPD) with one-third of all procedures, followed by interstitial lung disease (ILD) and CF as described elsewhere. In children, the overall leading diagnosis for lung transplantation is end-stage CF pulmonary disease; nevertheless, indications vary by age. In children less than 1 year of age, congenital heart disease (CHD) is the most frequent underlying disease. In children aged 1 to 10 years of age, end-stage CF pulmonary disease and idiopathic pulmonary arterial hypertension (IPAH) are the most frequent primary indications for lung transplants.

In older children and adolescents, CF is most common (5). It is important to emphasize that there are regional differences worldwide regarding primary indications leading to referral for pediatric lung transplantation, almost certainly reflecting diverse practices for referral, varying disease management and organ allocation rules for pediatric lung transplant candidates (5). In North America, half of pediatric lung transplants are carried out in children with end-stage pulmonary CF. In Europe on the other hand, over two-thirds of children undergoing lung transplantation suffer with CF (5) (*Figure 1*).

In children, lung re-transplants are rarely performed. Over the last two decades, only 100 pediatric lung re-transplant have been reported to the ISHLT Thoracic Transplant Registry, predominately from North America (8). Re-transplantation is predominantly undertaken in older children and adolescents and more frequently carried out beyond the first 12 months after primary transplantation. In most children, chronic lung allograft dysfunction (CLAD), primarily bronchiolitis obliterans syndrome (BOS), is the underlying cause leading to lung re-transplants (8).

Pediatric heart-lung transplantation is very rarely performed nowadays, with less than 10 procedures per year reported to the ISHLT Thoracic Transplant Registry in recent years (9). Heart-lung transplants in children are only performed in very few centers. The vast majority of pediatric heart-lung transplant procedures are undertaken in children with IPAH, in a minority in those children suffering with CHD (9).

In general, all children with end-stage parenchymal

and vascular pulmonary diseases on maximal medical treatment should be referred to a transplant program for lung transplant assessment if the predicted life expectancy is below 2 years (2,4). On the whole, the predicted life expectancy without lung transplantation has to be balanced with the expected post-transplant survival, taking into account the potential time on a waiting list, which could be particularly longer for children due to the general lack of suitable smaller donor organs. In Europe, children would most likely be referred to an adult transplant center with pediatric experience for transplant evaluation, in North America, generally to a pediatric transplant program. In order to maximize the net survival benefit of lung transplantation as the ultimate therapy option in children with advanced pulmonary disease, careful candidate selection is absolutely critical (2,4). The ISHLT Pulmonary Council has recently published an update on guidelines for referral and selection of lung transplant candidates, for the first time ever, including a general guidance on the pediatric lung transplant candidate selection (10). All in all it is important to note that no prospective, randomized studies have been conducted to date to support the published guidelines. Overall, timing of referral to a transplant center is similar in adult and pediatric practice, even though younger children should ideally be referred early as long waiting times for suitable smaller donor organs are to be expected (2,4). The updated ISHLT consensus document includes disease-specific criteria for referral and listing for transplant of the most common primary indications for lung transplantation. As CF is the most common pathology leading to pediatric lung transplantation and referral patterns and listing criteria are similar to adults, the consensus recommendations are discussed here in more detail. Patients with end-stage CF pulmonary disease should be referred for lung transplant assessment if on maximal medical therapy and in case of a forced expiratory volume in one second (FEV_1) <30% predicted, a 6-minute walk distance (6MWD) <400 m, pulmonary hypertension (outside a hypoxic exacerbation period) with a mean pulmonary arterial pressure (PAP) >25 mmHg measured invasively by right heart catheterization or a systolic PAP >35 mmHg on echocardiography, or other clinical signs of end-stage CF pulmonary disease such as a poor recovery from exacerbations, pneumothorax, life-threatening hemoptysis, or acute respiratory failure needing non-invasive ventilation (NIV) (10). Maximal medical therapy in patients with CF should ideally include a trial of newer CF therapies based on recent but advances in the field of CF research, therapies that have recently been introduced to the market modulating the basic defect in CF (11). Recent studies in highly selected CF patients using such disease modulators (potentiators/correctors) have shown promising clinical results, even in patients with advanced CF pulmonary disease or patients already listed for lung transplant (12). Therefore, patients with CF undergoing lung transplant evaluation should ideally be assessed for their eligibility of such CF-disease modulating therapies depending on their CF genotype. In selected lung transplant candidates, such disease modulating agents might lead to clinical stabilization, ideally prolonging the time of listing for transplant or stabilizing patients on the waiting list, all of which should aid to maximize net survival in CF patients.

In general, particularly young underweight females with CF and rapid pulmonary function decline should be referred early as this subgroup of CF patients has a poor prognosis. Then again, underweight body habitus itself may not in generally have a significant negative impact on survival of pediatric CF patients undergoing lung transplantation according to a recent ISHLT Thoracic Transplant Registry data analysis including over 800 pediatric lung transplant recipients (13). Listing of patients with CF for lung transplant is generally recommended in case of respiratory failure with hypoxia alone (PaO_2 <8 kPa or <60 mmHg) or hypercapnia ($PaCO_2$ >6.6 kPa or >50 mmHg), if requiring long-term NIV, rapid pulmonary function decline, frequent hospitalizations and/or WHO functional class IV (10).

At the assessment of every pediatric lung transplant candidate, the child and family require to be appropriately informed and sufficiently educated. Even a child should be willing to commit to the planned transplant operation and to generally consent to the close post-operative long-term follow-up needed. Child and family support is vital and should be implemented prior to listing for transplantation if not already set up (2,4).

Overall, adherence to medical treatment needs to be evaluated prior to listing for lung transplantation. Non-adherence is a leading cause for the development of CLAD and inferior long-term outcome post-transplant, in particular in adolescents, a well-known features following transplantation across all solid organ types (14).

As a general rule, contraindications in pediatric lung transplantation are similar to adult practice, but relative contraindications might be different between centers (4). In CF lung transplant candidates particularly acceptance of listing for lung transplant may differ among transplant programs, depending on CF airway pathogens isolated

prior to transplantation. Nevertheless, all transplant candidates have to be carefully assessed in view of potential infection risks (15). For CF transplant patients, some transplant programs have shown that patients chronically infected with *Burkholderia cenocepacia* have poorer post-transplant outcomes, but patients chronically infected with Burkholderia cepacia complex species other than *B. cenocepacia* do frequently have post-transplant survival comparable to other CF lung transplant recipients (16-19). Other airway important pathogens, such as nontuberculous mycobacteria (NTM) are frequently isolated in CF patients (up to 20%) referred for lung transplantation (20,21). Isolation of NTM in the airways of CF patients should not be considered an absolute contraindication for lung transplantation; however, all NTMs need to be classified first. In case of isolation of *M. abscessus* and depending on the antibiotic resistance pattern, some centers would consider such circumstance a contraindication for lung transplant. Nevertheless, appropriate treatment strategies needs to be discussed and implemented with support by the transplant infectious diseases specialist.

In pediatric lung transplant candidates with CF, the extra-pulmonary manifestations of CF have to be looked at carefully. In case of advanced CF-related liver disease, combined lung-liver transplantation needs to be evaluated. Other common extra-pulmonary disease manifestations of CF include diabetes mellitus, chronic rhino-sinusitis, CF-related bone disease and bowel problems such as recurrent episodes of distal intestinal obstruction syndrome (DIOS) (4).

Mechanical ventilation before lung transplantation has until recently been considered a contraindication for lung transplant in the majority of pediatric centers due to poor results previously reported (22). But newer data on the use of extracorporeal life/lung support (ECLS) prior to pediatric lung transplantation—particularly in awake patients—illustrate that pre-transplant ECLS might not generally lead to poor post-transplant outcomes if children are selected very carefully and treated at experienced centers (23-25).

Often smaller children wait a long time on the waiting list before a suitable donor organ is allocated, and in such candidates ECLS as a bridge to lung transplantation is to be considered. Children should be fully evaluated and already listed candidates on the transplant waiting list with rapidly advancing respiratory failure, to stabilize the child until a suitable donor organ is allocated. In general, candidates for ECLS as bridge to transplantation should be in single-organ failure with a good rehabilitation potential. The recently published consensus document by the ISLHT Pulmonary Council lists contraindication for ECLS as bridge to transplantation such as septic shock and multi-organ failure that are also applicable for pediatric candidates (10). A larger analysis of pediatric UNOS data on the use of ECMO at the time of lung transplantation showed no negative impact on the post-operative mortality rate (26). Further, newly published positive outcome results from Australia and Switzerland in small pediatric patient cohorts would promote the use of ECLS as a "bridge strategy" in highly selected, ideally awake, children (6,27). If pediatric patients are kept "awake" on ECLS prior to lung transplantation, physical deconditioning can be prevented as children have the possibility to get mobilized and perform physiotherapy, supporting post-operative recovery and rehabilitation (28-30). Thus, pre-transplant ECLS in children is nowadays considered as superior alternative to long-time mechanical ventilation by most centers, taking potential complications of the two methods into consideration. Nevertheless, more outcome data on pediatric lung transplant candidates on ECLS are needed to figure out key factors associated with poor post-operative results.

The technical details of the various forms of ECLS and possible complications using ECLS are beyond the scope of this chapter.

Donor acceptability criteria in pediatric lung transplantation

The ISHLT published donor acceptability criteria in the past, predominantly based on the adult lung transplant experience (31). The major limit of lung transplantation is the worldwide lack of suitable donor organs. Strategies to address the shortage of donor lungs include usage of so-called "marginal" donor organs (or extended criteria donor organs), organ donation after circulatory death (DCD) with or without ex-vivo lung perfusion (EVLP) as graft preparation pre-operatively, and graft size reduction (i.e., lobar lung transplants) (32,33). The latter is of particular interest in pediatric lung transplant candidates due to the shortage of donor organs for smaller children (34). Donor-recipient (D/R) size mismatch is an important aspect with a considerable influence on post-operative results (35-37). In oversized allografts, complications include atelectasis and segmental (or sub-segmental) airway distortion with impaired mucus clearance, a potential source of recurrent infections. Undersized allografts potentially cause persistent pneumothorax, graft hyperextension, compromised vital

lung capacity with reduced hemodynamic reserve, higher likelihood of primary graft dysfunction and the development of CLAD (38,39). In one of the largest pediatric data sets, the Zurich Group report on outcomes of 20 out of 29 children and adolescents after lung transplantation using size-reduced donor lungs due to pre-operative size-mismatches (40). No significant different short- and mid-term survival between the "full-size" and the "size-reduced bilateral transplant" patients were found.

Various centers described surgical techniques to successfully perform size-reduction of donor lung grafts in children; details are beyond the scope of this chapter. The Zurich Group also published a case report of a simultaneously performed bilateral lobar lung transplant derived from one donor into two small adolescents with CF (41). Both patients have an unimpaired lung function even more than five years post-transplant with no evidence of CLAD. However, such an approach is not routinely performed to overcome donor shortage in smaller lung transplant recipients.

Management of pediatric lung transplant recipients and post-transplant outcome

Immunosuppressive treatment is the keystone to prevent lung allograft rejection (42). In general, the majority of children undergoing lung transplantation receive induction therapy, most commonly an interleukin-2 receptor antagonist (5,43). Similar to adults, children generally are on maintenance triple immunosuppression post-transplant (cyclosporine/tacrolimus, mycophenolate mofetil, steroids). Nowadays, tacrolimus is more commonly prescribed than cyclosporine. Pediatric programs cooperating within the International Pediatric Lung Transplant Collaborative (IPLTC) have recently agreed upon an immunosuppressant treatment protocol for children undergoing lung transplantation that includes tacrolimus, mycophenolate mofetil and prednisolone (Samuel Goldfarb, personal communication).

Although graft function is to be preserved, immunosuppressant-related side effects are common in children following lung transplantation. Therefore, strategies need to be put into standard practice to reduce immunosuppression-related side effects such as nephrotoxicity through careful therapeutic drug monitoring and lowering of target levels of calcineurin-inhibitors, to avoid acute-reversible and chronic-irreversible renal impairment (44). Instead of a "one fits all approach",

tailored immunosuppression and a personalized therapy approach is to be advocated, particularly in children with a good lung allograft function and no evidence of CLAD (4).

Further, infectious complications are common causes for morbidity and mortality in pediatric lung transplant recipients, accounting for almost 50% of death during the first twelve months after transplantation (5). Children following lung transplantation that are at a high risk for infections caused by cytomegalovirus (CMV)—defined as positive recipient or donor serology—get CMV prophylaxis (45). However, current practice among pediatric programs varies, even though international consensus guidelines—but not specifically for children—on the management of CMV in solid organ transplant recipients were published (46). Furthermore, fungal lung infections are not uncommon, and recently published pediatric data show a decreased 12-month post-transplant survival (47). Respiratory viral infections are very common after lung transplantation in children, sources are often siblings or peer groups and associated with a decreased 1-year survival (48). In order to at least reduce the burden of vaccine preventable diseases, children should be vaccinated prior to be placed on the transplant waiting list. It is well known that vaccinations are frequently incomplete before transplantation in these children. National vaccination guidelines should be followed; vaccinations guidelines for pediatric lung transplant patients are center-specific, no consensus guidelines exist to the author's knowledge. Vaccinations of household contacts are also highly recommended (49).

Overall, survival after pediatric lung transplantation is comparable to adults (5,50). As in adult lung transplantation, survival following pediatric lung transplantation has improved over time, primarily due to superior early post-operative survival, i.e., better surgical techniques and early post-operative intensive care management (2,4).

According to the most recent ISHLT Thoracic Transplant Registry Report, 5-year survival after lung was 53% in the recent era [2002–2009] (5). Individual pediatric programs have published survival data exceeding the results of the ISHLT Registry (6). The Zurich Group recently put out its contemporary data on lung transplantation in children and adolescents (up to 20 years of age), quoting an overall 5-year survival over 75% in predominantly patients with CF (6). However, it remains somewhat difficult to predict survival following lung transplantation in children with advanced CF pulmonary disease. Various studies in adults have been published demonstrating a survival benefit

in adult lung transplantation for CF. But in children with CF, survival data following lung transplantation are mostly based on single-center reports (51,52). However, a report from Zurich clearly illustrates a true survival benefit in 80 CF patients undergoing lung transplantation, with no negative impact of pediatric age (<18 years of age) on post-transplant survival (53). In the study by Hofer *et al.*, estimated 5-year survival without transplant was 33% compared to a 5-year post-transplant survival of 67%. On the other hand, Liou and co-workers concluded based on UNOS data that lung transplantation would not improve survival in children with CF (54). Further, Liou and co-workers published a controversially discussed analysis in 2007 using US Cystic Fibrosis Foundation Patient Registry and Organ Procurement and Transplantation Network (OPTN) data on patients undergoing lung transplantation between 1992 and 2002 to demonstrate that only five of over 500 CF children awaiting lung transplantation can expect an improved survival (55). Various authors discussed future directions of studies investigating survival benefit of children with CF undergoing lung transplantation following publication of the study by Liou *et al.*; however, data are still lacking (56,57).

As in adult lung transplantation, the development of CLAD is the major hurdle to achieve better overall survival also after lung transplantation in children. BOS, the most common form of CLAD, is the leading cause of death (>40%) by 5 years following transplantation. Similar to adults, further, around half of the surviving children develop BOS by 5 years after lung transplantation (5). On the other hand, overall functional status of children surviving lung transplantation is reported to be good.

As described elsewhere in detail, international clinical practice guidelines for the diagnosis and management of BOS have recently been published (58). To date, no well-proven therapy approach exists to successfully manage CLAD in lung transplant recipients, both in adults and children. Similar attempts include change/augmentation of immunosuppression, and use of macrolides, extracorporeal photopheresis (ECP), and total lymphoid irradiation (TLI) (59). Pediatric data do generally not exist. The final option for advanced lung allograft failure is lung re-transplantation. As described above, data on pediatric lung re-transplantation is limited, most cases performed for CLAD-BOS, predominantly in older children (8). Based on limited published outcome data, pediatric lung re-transplants appear to be more successful if re-transplantation follows a minimum of twelve months after primary transplantation and in children not

mechanically ventilated at time of re-transplantation (60). Ideally, pediatric candidates for lung re-transplantation should have no second organ failure.

Lastly, with improvements in pediatric lung transplantation detailed above, transition from pediatric to adult care is more frequent. As transition is not a single event but rather a process of an adolescent lung transplant recipient being transferred to adult care providers, varies aspects of other transitions of an adolescent that take place simultaneously need to be looked at (i.e., autonomy, self-identity, cognition, sexuality, physical appearance, education) (61). Ideally, transition should advocate self-care and decision-making of the adolescent, but also include parents/caregivers, taking into account the adolescent's chronological age, physical and cognitive maturity. In general, transition is regarded of interest for both pediatric and adult transplant care teams (2,4).

To sum up, pediatric lung transplants have successfully been carried out in children of all age groups, including infants, with encouraging outcomes. Similar to adult lung transplantation, the development of CLAD remains the burden of lung transplantation in children and adults restricting long-term success. Potential pediatric candidates for lung transplantation should be referred early, assessed thoroughly and selected very carefully in order to maximize the overall net survival benefit following pediatric lung transplantation. Specific pediatric aspects of lung transplantation are the shortage of suitable donor organs for smaller children and psychosocial aspects and adherence, in particular, in adolescents.

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Footnote

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References

1. Mendeloff EN. The history of pediatric heart and lung transplantation. Pediatr Transplant 2002;6:270-9.
2. Benden C. Specific aspects of children and adolescents undergoing lung transplantation. Curr Opin Organ Transplant 2012;17:509-14.
3. Hayes D Jr, Benden C, Sweet SC, et al. Current state of

pediatric lung transplantation. Lung 2015;193:629-37.

4. Schmid FA, Benden C. Special considerations for the use of lung transplantation in pediatrics. Expert Rev Respir Med 2016;10:655-62.

5. Goldfarb SB, Levvey BJ, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: Nineteenth pediatric lung and heart-lung transplantation report – 2016; focus theme: primary diagnostic indications for transplant. J Heart Lung Transplant 2016;35:1196-205.

6. Schmid FA, Inci I, Bürgi U, et al. Favorable outcome of children and adolescents undergoing lung transplantation at a European adult center in the new era. Pediatr Pulmonol 2016;51:1222-8.

7. Hayes D Jr, Sweet SC, Benden C, et al. Transplant center volume and outcomes in lung transplantation for cystic fibrosis. Transpl Int 2017;30:371-7.

8. Benden C, Goldfarb SB, Edwards LB, et al. The rregistry of the International Society for Heart and Lung Transplantation: seventeenth official pediatric lung and heart-lung transplantation report – 2014; focus theme: retransplantation. J Heart Lung Transplant 2014;33:1025-33.

9. Benden C, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: fifteenth pediatric lung and heart-lung transplantation report – 2012. J Heart Lung Transplant 2012;31:1087-95.

10. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.

11. Bosch B, De Boeck K. Searching for a cure for cystic fibrosis. A 25-year quest in a nutshell. Eur J Pediatr 2016;175:1-8.

12. Elborn JS, Ramsey BW, Boyle MP, et al. Efficacy and safety of lumacaftor/ivacaftor combination therapy in patients with cystic fibrosis homozygous for Phe508del CFTR by pulmonary function subgroup: a pooled analysis. Lancet Respir Med 2016;4:617-26.

13. Benden C, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Sixteenth Official Pediatric Lung and Heart-Lung Transplantation Report – 2013; focus theme: age. J Heart Lung Transplant 2013;32:989-97.

14. Simons LE, Blount RL. Identifying barriers to medication adherence in adolescent transplant recipients. J Pediatr Psychol 2007;32:831-44.

15. Luong ML, Morrissey O, Husain S. Assessment of infection risks prior to lung transplantation. Curr Opin Infect Dis 2010;23:578-83.

16. Murray S, Charbeneau J, Marshall BC, et al. Impact of burkholderia infection on lung transplantation in cystic fibrosis. Am J Respir Crit Care Med 2008;178:363-71.

17. Alexander BD, Petzold EW, Reller LB, et al. Survival after lung transplantation of cystic fibrosis patients infected with Burkholderia cepacia complex. Am J Transplant 2008;8:1025-30.

18. Hopkins PM, Kidd TJ, Coulter C, et al. Death after lung transplantation in cystic fibrosis patients infected with Burkholderia cepacia. Am J Respir Crit Care Med 2009;179:257-8.

19. De Soyza A, Meachery G, Hester KL, et al. Lung transplantation for patients with cystic fibrosis and Burkholderia cepacia complex infection: a single-center experience. J Heart Lung Transplant 2010;29:1395-404.

20. Olivier KN, Weber DJ, Wallace RJ, et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. Am J Respir Crit Care Med 2003;167:828-34.

21. Chalermskulrat W, Sood N, Neuringer IP, et al. Non-tuberculous mycobacteria in end stage cystic fibrosis: implications for lung transplantation. Thorax 2006;61:507-13.

22. Elizur A, Sweet SC, Huddleston CB, et al. Pre-transplant mechanical ventilation increases short-term morbidity and mortality in pediatric patients with cystic fibrosis. J Heart Lung Transplant 2007;26:127-31.

23. Cypel M, Keshavjee S. Extracorporeal life support as a bridge to lung transplantation. Clin Chest Med 2011;32:245-51.

24. Hoopes CW, Kukreja J, Golden J, et al. Extracorporeal membrane oxygenation as a bridge to pulmonary transplantation. J Thorac Cardiovasc Surg 2013;145:862-7.

25. Inci I, Klinzing S, Schneiter D, et al. Outcome of extracorporeal membrane oxygenation as a bridge to lung transplantation: An institutional experience and literature review. Transplantation 2015;99:1667-71.

26. Hayes D Jr, McConnell PI, Tobias JD, et al. Survival in children on extracorporeal membrane oxygenation at the time of lung transplantation. Pediatr Transplant 2015;19:87-93.

27. Casswell GK, Pilcher DV, Martin RS, et al. Buying time: The use of extracorporeal membrane oxygenation as a bridge to lung transplantation in pediatric patients. Pediatr Transplant 2013;17:E182-8.

28. Olsson KM, Simon A, Strueber M, et al. Extracorporeal membrane oxygenation in nonintubated patients as bridge to lung transplantation. Am J Transplant 2010;10:2173-8.

29. Garcia JP, Iacono A, Kon ZN, et al. Ambulatory extracorporeal membrane oxygenation: a new approach for bridge-to-lung transplantation. J Thorac Cardiovasc Surg 2010:139;e137-9.

30. Schmidt F, Sasse M, Boehne M, et al. Concept of "awake venovenous extracorporeal membrane oxygenation" in pediatric patients awaiting lung transplantation. Pediatr Transplant 2013;17:224-30.

31. Orens JB, Boehler A, de Perrot M, et al. A review of lung transplant donor acceptability criteria. J Heart Lung Transplant 2003;22:1183-200.

32. Aigner C, Winkler G, Jaksch P, et al. Size-reduced lung transplantation: an advanced operative strategy to alleviate donor organ shortage. Transplant Proc 2004;36:2801-5.

33. Santos F, Lama R, Alvarez A, et al. Pulmonary tailoring and lobar transplantation to overcome size disparities in lung transplantation. Transplant Proc 2005;37:1526-9.

34. Mueller C, Hansen G, Ballmann M, et al. Size reduction of donor organs in pediatric lung transplantation. Pediatr Transplant 2010;14:364-8.

35. Inci I, Schuurmans MM, Kestenholz P, et al. Long-term outcomes of bilateral lobar lung transplantation. Eur J Cardiothorac Surg 2013;43:1220-5.

36. Aigner C, Mazhar S, Jaksch P, et al. Lobar transplantation, split lung transplantation and peripheral segmental resection – reliable procedures for downsizing donor lungs. Eur J Cardiothorac Surg 2004;25:179-83.

37. Eberlein M, Reed RM, Permutt S, et al. Parameters of donor-recipient size mismatch and survival after bilateral lung transplantation. J Heart Lung Transplant 2012;31:1207-13.e7.

38. Eberlein M, Permutt S, Chahla MF, et al. Lung size mismatch in bilateral lung transplantation is associated with allograft function and bronchiolitis obliterans syndrome. Chest 2012;141:451-60.

39. Eberlein M, Reed RM, Bolukbas S, et al. Lung size mismatch and primary graft dysfunction after bilateral lung transplantation. J Heart Lung Transplant 2015;34:233-40.

40. Benden C, Inci I, Weder W, et al. Size-reduced lung transplantation in children – an option worth to consider. Pediatr Transplant 2010;14:529–33.

41. Inci I, Benden C, Kestenholz P, et al. Simultaneous bilateral lobar lung transplantation: one donor serves two recipients. Ann Thorac Surg 2013;96:e69-71.

42. Coelho T, Tredger M, Dhawan A. Current status of immunosuppressive agents for solid organ transplantation in children. Pediatr Transplant 2012;16: 106-22.

43. Hayes D Jr, Kirkby S, Wehr AM, et al. A contemporary analysis of induction immunosuppression in pediatric lung transplant recipients. Transpl Int 2014;27:211-8.

44. Sarwal M, Pascual J. Immunosuppression minimization in pediatric transplantation. Am J Transplant 2007;7:2227-35.

45. Danziger-Isakov LA, Worley S, Michaels MG, et al. The risk, prevention, and outcome of cytomegalovirus after pediatric lung transplantation. Transplantation 2009;87:1541-8.

46. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. Transplantation 2010;89:779-95.

47. Liu M, Worley S, Mallory GB Jr, et al. Fungal infections in pediatric lung transplant recipients: colonization and invasive disease. J Heart Lung Transplant 2009;28:1226-30.

48. Liu M, Worley S, Arrigain S, et al. Respiratory viral infections within one year after pediatric lung transplant. Transpl Infect Dis 2009;11:304-12.

49. Benden C, Danziger-Isakov LA, Astor T, et al. Variability in immunization guidelines in children before and after lung transplantation. Pediatr Transplant 2007;11:882-7.

50. Zafar F, Heinle JS, Schecter MG, et al. Two decades of pediatric lung transplant in the United States: have we improved? J Thorac Cardiovasc Surg 2011;141:828-32.

51. Görler H, Strüber M, Ballmann M, et al. Lung and heart-lung transplantation in children and adolescents: a long-term single center experience. J Heart Lung Transplant 2009;28:243-8.

52. Gruber S, Eiwegger T, Nachbaur E, et al. Lung transplantation in children and young adults: a 20 years single center experience. Eur Respir J 2012;40:462-9.

53. Hofer M, Benden C, Inci I, et al. True survival benefit of lung transplantation for cystic fibrosis patients: the Zurich experience. J Heart Lung Transplant 2009;28:334-9.

54. Liou TG, Adler FR, Huang D. Use of lung transplantation survival models to refine patient selection in cystic fibrosis. Am J Respir Crit Care Med 2005;171:1053-9.

55. Liou TG, Adler FR, Cox DR, et al. Lung transplantation and survival in children with cystic fibrosis. N Engl J Med 2007;357:2143-52.

56. Sweet SC, Aurora P, Benden C, et al. Lung transplantation and survival in children with cystic fibrosis: solid statistics – flawed interpretation. Pediatr Transplant 2008;12:129-36.

57. Sweet SC, Benden C, Elidemir O. Lung transplantation and survival in children with cystic fibrosis. N Engl J Med

2008;358:1754.

58. Meyer KC, Raghu G, Verleden GM, et al. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J 2014;44:1479-503.

59. Benden C, Danziger-Isakov L, Faro A. New developments in treatment after lung transplantation. Curr Pharm Des

2012;18:737-46.

60. Scully BB, Zafar F, Schecter MG, et al. Lung retransplantation in children: appropriate when selectively applied. Ann Thorac Surg 2011;91:574-9.

61. LaRosa C, Glah C, Baluarte HJ, et al. Solid-organ transplantation in childhood: transitioning to adult health care. Pediatrics 2011;127:742-53.

Living-related lung transplantation

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Abstract: Living-donor lobar lung transplantation (LDLLT) was developed to deal with the severe shortage of brain dead door for patients who would not survive the long waiting period. In standard LDLLT, right and left lower lobes removed from two healthy donors are implanted into a recipient after right and left pneumonectomies using cardiopulmonary bypass (CPB). The number of LDLLT has decreased in the USA due to the recent change in allocation system for cadaveric donor lungs. For the past several years, most of the reports on LDLLT have been from Japan, where the average waiting time for a cadaveric lung is exceeding 800 days. LDLLT has been performed both for adult and pediatric patients suffering from various end-stage lung diseases including restrictive, obstructive, vascular and infectious lung diseases. Since only two lobes are implanted, size matching is a very important issue. Functional size matching by measuring donor pulmonary function and anatomical size matching by three-dimensional computed tomography (3D-CT) volumetry are very useful. For oversize graft, we have employed several techniques, including single lobe transplantation, delayed chest closure, downsizing the graft, and middle lobe transplantation. In cases of undersize mismatch, native upper lobe sparing transplant or right-left inverted transplant was performed. The 5-, 10- and 15-year survivals were 80.8%, 72.6% and 61.7%, respectively. There was no difference in survival between standard LDLLT and non-standard LDLLT such as single, sparing and inverted transplant. All donors have been discharged without any restrictions. LDLLT is a viable option for very ill patients who would not survive a long waiting time for cadaveric lungs. We have successfully developed various surgical techniques to overcome size mismatching with favorable outcome.

Keywords: Lung transplantation; cadaveric lung transplantation (CLT); living-donor lobar lung transplantation (LDLLT); size matching

Introduction

To deal with the brain-dead donor shortage, living-donor lobar lung transplantation (LDLLT) was first developed in the USA as an alternative modality for very sick patients who would not survive a waiting time for cadaveric lung transplantation (CLT) (1). In a standard LDLLT, the right and left lower lobes from two healthy donors are implanted into the recipient after right and left pneumonectomies (*Figure 1*). In the beginning, the procedure was applied to pediatric or small adult patients with cystic fibrosis (CF) (2). It is now well known that LDLLT can be applied to various indications including restrictive, obstructive, infectious and vascular diseases (3-7). Although LDLLT was developed in the USA, its use has decreased there due to the recent change in lung allocation system. For the past several years, most of the reports on LDLLT have been from Japan, where the average waiting time for a cadaveric lung is exceeding 800 days. Besides Japanese experience, England (8), Brazil (9) and China (10) have reported their small practices. We have reported that bilateral LDLLT provides equal or better survival than conventional CLT (11).

Recipient selection

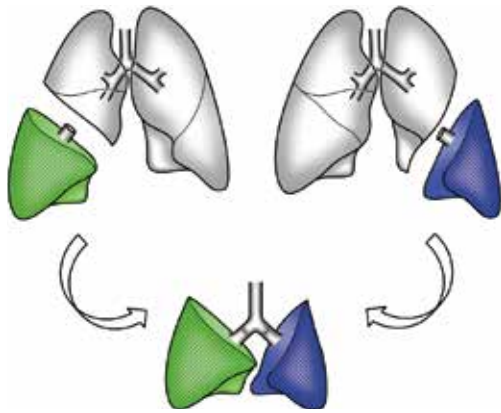Recipient candidates for LDLLT should be less than 65

Figure 1 Bilateral living-donor lobar lung transplantation. Right and left lower lobes from two healthy donors are implanted in a recipient in place of whole right and left lungs, respectively.

years old and must meet the criteria for conventional CLT. Our policy has been to limit LDLLT to severely ill patients with rapidly progressive lung disease who would not survive the long waiting time for cadaveric lungs. At the time of transplantation, all of our LDLLT recipients were oxygen dependent and 59% of them were bed bound and 11% of them were on a ventilator at the time of transplantation. It should be also noted that it would not be justified to do two lobectomies from two healthy relatives if the recipient has too many risk factors. It has been debated if LDLLT can be indicated for patients already on a ventilator or requiring re-transplantation. In St. Louis group experience, survival was better after LDLLT than conventional CLT for re-transplantation (12). Perioperative mortality of re-transplantation was only 7.7% after LDLLT versus 42.3% after CLT. USC group reported that ventilator dependency and re-transplantation were significant risk factors of death in their 123 LDLLTs (13). In our experience, all 14 patients on a ventilator for as long as 7 months underwent successful LDLLT.

CF is the most frequent indication for LDLLT in USA. This is because only two lobar grafts are implanted and CF patients are usually small in body size. CF is very rarely seen in Japan where the distribution of diagnoses is quite unique as compared with USA (14). Various lung diseases including restrictive, obstructive, infectious and vascular lung diseases have been accepted for LDLLT candidate in our experience. Among them three major indications were, interstitial pneumonia, bronchiolitis obliterans, and pulmonary hypertension. Majority of the patients with

interstitial pneumonia were on systemic steroid therapy (6). Major cause of bronchiolitis obliterans was graft-versus-host disease after hematopoietic stem cell transplantation for hematologic disorders such as leukemia (15). High dose epoprostenol therapy had been already given to patients with idiopathic pulmonary arterial hypertension (7). Although knowledge of the survival predictors in each disease is helpful, ultimately the timing of LDLLT must be decided base on the unique situation of each patient.

Donor selection

Table 1 summarizes the eligibility criteria for living lobar lung donation at Kyoto University. We have accepted only immediate family members (relatives within the third degree or a spouse) in our institution. However, extended family members and unrelated individuals have been accepted in other non-Japanese institutions (16). It should be prohibited to extract more than one lobe from one donor.

It is very important to confirm that potential donors are competent, willing to donate without psychologic pressure from the others. They should be medically and psychosocially suitable. We inform them about the risks and benefits as a donor, and also inform them about the risks, benefits, other possible treatment option of the recipient. We interview potential donors at least 3 times to provide them what is called "cooling-off" period.

Regarding preoperative workup, posterior-anterior and lateral chest X-ray, enhanced high-resolution computed tomography (CT) of the chest, pulmonary function tests, arterial blood gases, electrocardiogram, and Doppler echocardiogram are performed. Three-dimensional multidetector CT angiography is created for the confirmation of the pulmonary arterial and venous anatomy (*Figure 2*) (17). The completeness of inter pulmonary fissures is carefully evaluated by high-resolution CT. Although human leukocyte antigens (HLA) matching is not required for donor selection, we perform a prospective cross-match to rule-out the presence of anti-HLA antibodies.

We usually select the larger donor with better vital capacity for the right-side donor and select the other for the left-side donor.

Size matching

Because only two lobes are implanted in standard LDLLT, it is very important to evaluate size matching between the

Table 1 The eligibility criteria for living lung donation (Kyoto University)

Medical criteria
Age 20–60 years
ABO blood type compatible with recipient
Relatives within the third degree or a spouse
No significant past medical history
No recent viral infection
No significant abnormalities on echocardiogram and electrocardiogram
No significant ipsilateral pulmonary pathology on computed tomography
Arterial oxygen tension ≥80 mmHg (room air)
Forced vital capacity, forced expiratory volume in one second ≥85% of predicted
No previous ipsilateral thoracic surgery
No active tobacco smoking
Social and ethical criteria
No significant mental disorders proved by a psychiatrist
No ethical issues or concerns about donor motivation

Figure 2 Three-dimensional computed tomography angiography in a left donor. A yellow-dotted line shows the planned cutting oblique line of the pulmonary artery, thus to preserve ligula branches.

donor and recipient. We often implant relatively small grafts in LDLLT in which only two lobar grafts are implanted. However, excessively small grafts may result in lung edema with high pulmonary vascular resistance (18). Dead chest cavity may cause persistent air leakage and empyema. Overexpansion of the donor grafts may lead to obstructive physiology by small airway closure (19).

For small children under the height of 100 cm, the adult lower lobe is usually too big. It may not be possible to close the chest after implanting excessively oversized grafts, because chest closure could increase airway resistance, atelectasis and hemodynamic instability (20).

Functional size matching

We mainly use the forced vital capacity (FVC) size matching to evaluate undersized grafts. Pulmonary function can be measured in living-donor but not in cadaveric-donor. It allows us to perform more precise functional matching in LDLLT. For "functional size matching", we rely on graft FVC (4,21). We have made a formula for estimating the graft FVC based on the donor's measured FVC and the number of pulmonary segments implanted. Total FVC of the two grafts can be calculated by the following equation given that the right lower lobe consists of 5 segments, the left lower lobe of 4 and the whole lung of 19.

Total FVC of the two grafts = measured FVC of the right donor ×5/19 + measured FVC of the left donor ×4/19.

Our acceptable lower threshold of the total FVC of the two grafts is 45% of the predicted FVC of the recipient (calculated based on height, age, and sex). We think that the ratio should be more than 50% for recipients with pulmonary vascular diseases such as pulmonary hypertension.

Total FVC of the two grafts/predicted FVC of the recipient >0.45–0.50.

Anatomical size matching

We mainly use the volumetric size matching to evaluate oversized grafts. For "anatomical size matching", three-dimensional CT (3D-CT) volumetry is performed both for the donor and the recipient (22). CT images are obtained using a multi-detector CT scanner during a single respiratory pause at the end of maximum inspiratory effort. Contiguous 0.5-mm slices, reconstructed using a standard lung reconstruction algorithm, are used for volumetric analysis and the entire CT image is exported to

Anatomical size matching
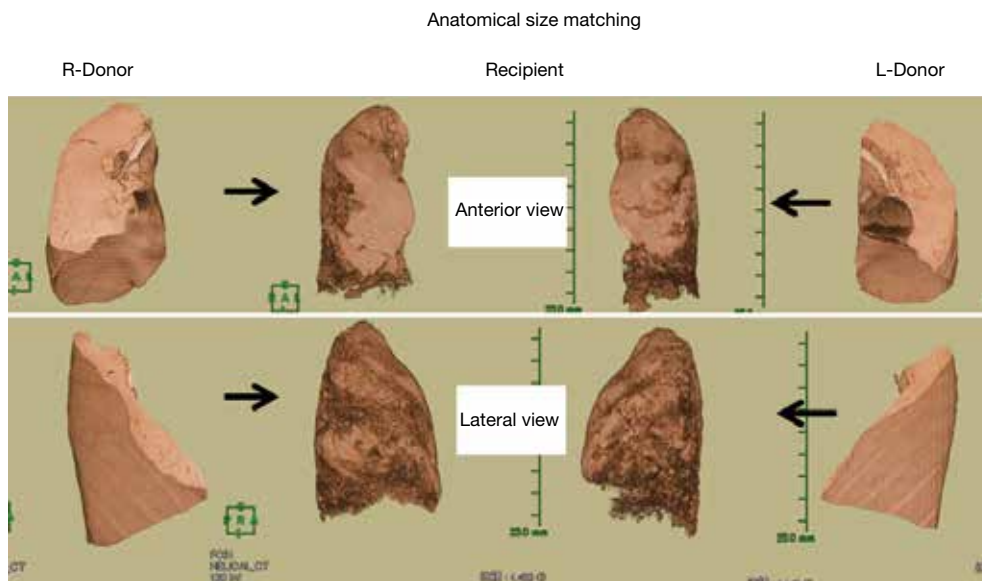
Figure 3 Anatomical size matching for the donor grafts and the recipient thorax using three-dimensional volumetry. The recipient was an adult male with pulmonary fibrosis. His right and left hemithorax was 1,483 and 1,149 mL, respectively. The right donor was a male whose right lower lobe was 1,637 mL. The left donor was a female whose left lower lobe was 716 mL. The right graft was oversize (110%) and the left graft was undersize (62.3%). Uneventful bilateral living-donor lobar lung transplantation was performed.

a workstation (AZE Virtual Place Lexus; AZE Co., Ltd., Tokyo, Japan) for 3D-CT volumetry (*Figure 3*). Using automated segmentation, the volumes of each lung and the graft lobes are calculated automatically. The upper and lower thresholds of anatomical size matching have not been precisely determined yet. We have accepted a wide range of volume ratios between the donor's lower lobe graft and the corresponding recipient's chest cavity. The upper threshold of the volume ratio appears to be about 200% based on 3D-CT size matching.

Surgical technique

Performing bilateral LDLLT requires a lot of man power for three surgical teams and a back-table team. We communicate each other closely to identify appropriate timing for graft extraction to minimize graft ischemic time. We usually bring the recipient and the right-side donor to operating room (OR) at the same time followed by bringing the left-side donor 30 min later.

Donor lobectomy

Because heparin is used in the donor, we usually place

an epidural catheter the day before the operation for postoperative pain management. Donors are intubated with a left-sided double lumen endotracheal tube under general anesthesia. With lateral decubitus position, a posterolateral thoracotomy is made though the 5th intercostal space. At first, interlobar fissures are divided by linear stapling devices. It is important to open pericardium near the inferior pulmonary vein circumferentially. In the interlobar fissure, pulmonary artery branches are to be dissected carefully. It is important to define the anatomy of the pulmonary artery branches to middle lobe and the lower lobe in the right-side donor. In the left-side donor, the anatomy of the pulmonary arteries to the lingular segment should be carefully evaluated. When the branches of middle lobe artery and lingular artery are small, they could be ligated and sacrificed. When such branches are large, we try to preserve them by arterioplasty using autopericardial patch (23,24).

When the recipient's pneumonectomy is nearly completed, intravenous prostaglandin E1 is administered with a dose of decreasing a systolic blood pressure by 10 to 20 mmHg. Then, 5,000 units of heparin and 500 mg of methylprednisolone are given intravenously. We place a vascular clamp on the interlobar pulmonary artery followed

by placing another vascular clamp on the inferior pulmonary vein intrapericardially. The pulmonary vein, the pulmonary artery and bronchus are divided in this order. Vascular stamps are sutured with 5-0 polypropylene running suture. The bronchus is closed with 4-0 polypropylene interrupted sutures and covered with pedicled pericardial fat tissue.

At the back table, the lobar grafts are flushed with preservation solution (ET-Kyoto solution in our institution) both antegradely and retrogradely. We ventilate the lobar grafts gently with room air throughout the flush.

Recipient implantation

When a recipient is very unstable, we dissect right femoral vessels under local anesthesia just in case for urgent vascular access. Adult recipients are intubated with a left-side double lumen endotracheal tube under general anesthesia. For children and small adults, a single lumen endotracheal tube is used. Both chest cavities are entered through the 4th intercostal space by means of "clamshell" incision. To facilitate postoperative sternal fixation, the sternum is notched at the level of transection.

We at first perform pleural and hilar dissections as much as possible before heparinization, which would reduce blood loss. Regarding intraoperative circulatory support, we have utilized extracorporeal membrane oxygenation (ECMO) instead of conventional cardiopulmonary bypass (CPB) since 2012. We try to minimize heparin administration and aim to maintain activated clotting time between 180 to 200 seconds. Two drainage cannulas are placed, one to the right atrium via the right femoral vein, the other to the superior vena cava via the right appendage. These two drainage cannulas are connected by a Y connector. The ascending aorta is also cannulated for blood feeding.

After right pneumonectomy, the right lower lobe graft is implanted. The bronchus, the pulmonary vein, and the pulmonary artery are anastomosed in this order. The bronchial anastomosis is performed with a running 4-0 polydioxanone suture for membranous portion and with simple interrupted sutures for cartilaginous portion. When the bronchial size is similar between the recipient main bronchus and the donor lobar bronchus, we use end-to-end anastomosis. Telescoping technique is used when the discrepancy in bronchial size is obvious. No bronchial wrapping is employed. The pulmonary venous anastomosis is performed between the donor inferior pulmonary vein and the recipient upper pulmonary vein using a running 6-0 polypropylene suture. The venous suture is left untied for

the subsequent deair procedure at the time of reperfusion. Lastly, the pulmonary arterial anastomosis is conducted in an end-to-end fashion using a running 6-0 polypropylene suture. Just before completing the right graft implantation, 500 mg of methylprednisolone is given intravenously and nitric oxide inhalation is initiated at 20 ppm. Antegrade reperfusion of the graft is done by releasing the vascular clamp on the pulmonary artery. We discard preservation solution and some amount of blood through the untied venous anastomosis to ensure the deair. After the right graft is reperfused and ventilated, ECMO flow is gradually decreased to about 70% of the full flow to maintain adequate blood flow to the first implanted right graft.

Left pneumonectomy and left graft implantation are conducted in the same manner. After the both lungs are reperfused and ventilated, ECMO is gradually weaned to the level of 10% of the full flow. When the blood gas and hemodynamics are satisfactory, ECMO is removed.

Strategies for size mismatch in LDLLT

Oversized graft

An adult lobe could be too big for a small child. We have employed several compensatory techniques such as single lobe transplantation with or without contralateral pneumonectomy, delayed chest closure, downsizing the graft, and implanting middle lobe. Single LDLLT is also indicated when only one living-donor is found in the family. We previously reported acceptable results after single LDLLT for very sick patients. However, when two donors were available, bilateral LDLLT provided better outcome (25).

Undersized graft

For large adults, two lower lobe grafts may be too small. We have developed two transplant procedures, native upper lobe sparing LDLLT (26) and right-left inverted LDLLT (27).

Native upper lobe sparing LDLLT (*Figure 4*) is indicated when the total graft FVC is less than 60% of the recipient's predicted FVC. The recipient lung should not be infected and the interlobar fissure should be well developed. Ideally, the native upper lobes are less impaired than the lower lobes as seen on high-resolution CT or are better perfused on perfusion scintigraphy. The surgical procedure of native upper lobe sparing transplant is similar to that of standard LDLLT except that the bronchus is anastomosed distally to the second carina, the pulmonary vein to the lower
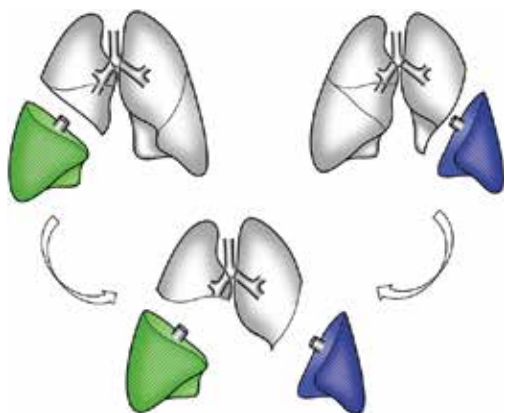
Figure 4 Native upper lobe sparing living-donor lobar lung transplantation. Bilobectomy and left lower lobectomy are performed in the recipient, and lower lobar grafts are implanted.
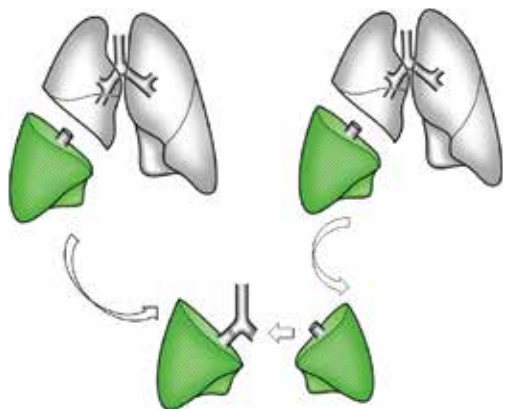

Figure 5 Right-to-left inverted living-donor lobar lung transplantation. The donor right lower lobe (5 segments) is inverted and implanted into the recipient's left chest cavity instead of the donor left lower lobe (4 segments).

pulmonary vein and the pulmonary artery to the interlobar artery in the fissure.

In right-left inverted LDLLT (*Figure 5*), the donor right lower lobe (5 segments) is inverted and implanted into the recipient's left chest cavity instead of the donor left lower lobe (4 segments). It is usually indicated when total graft FVC is less than 60% of the recipient's predicted FVC or when donor's left lower lobectomy would be technically difficult due to interlobar pulmonary artery anatomy. The technical details have been described previously. At the time of left pneumonectomy in the recipient, upper and lower bronchi are stapled separately. After rotating the right lower lobe graft from its anatomic

position to 180° about its superior-inferior axis, the graft is placed in the recipient's left chest cavity. The bronchus is anastomosed to the recipient's left upper bronchus and the left lower bronchial stamp is left closed. The pulmonary artery anastomosis is performed behind the bronchus. The donor pulmonary vein is anastomosed to the recipient's left upper pulmonary vein or occasionally to the recipient's left appendage.

Nearly half of our LDLLTs were performed as non-standard LDLLT using single, sparing or inverted techniques. Most of those patients would not have been accepted if the aforementioned new techniques were not used. The survival after non-standard LDLLT were similar to survival rates after standard LDLLT (28).

Postoperative management

Meticulous postoperative management is required in ICU. We usually keep the patient intubated for more than 3 days to maintain optimal expansion of the implanted lobar grafts. Pressure-limited ventilation is used and maximal ventilation pressure is kept less than 25 cmH$_2$O. Fiberoptic bronchoscopy is performed twice a day when intubated to assess bronchial anastomoses and to clean retained secretions by suction. Aggressive bedside rehabilitation is initiated as soon as possible.

Triple drug therapy with cyclosporine (CSA) or tacrolimus (FK), mycophenolate mofetil (MMF), and corticosteroids are used for postoperative immunosuppression. We do not use induction cytolytic therapy. Methylprednisolone (125 mg) is administered intravenously during the first 3 days. We give all other immunosuppressants via the nasal tube inserted to the proximal jejunum to protect renal function.

We do not perform routine transbronchial lung biopsy for rejection monitoring. It is because the risk of pneumothorax and bleeding after transbronchial lung biopsy may be greater after LDLLT. Acute rejection can be judged on the basis of careful monitoring radiographic and clinical findings. It is unique that acute rejection is usually seen as unilateral pulmonary infiltration on chest radiographs and CT after LDLLT because two lobes are donated by different donors. Clinical findings of early acute rejection include dyspnea, low grade fever, leukocytosis, hypoxemia. When acute rejection is suspected, we administer a trial bolus dose of methylprednisolone 500 mg and carefully observe various clinical signs. If the patient improves after the first dose of methylprednisolone, two additional daily bolus doses are given.
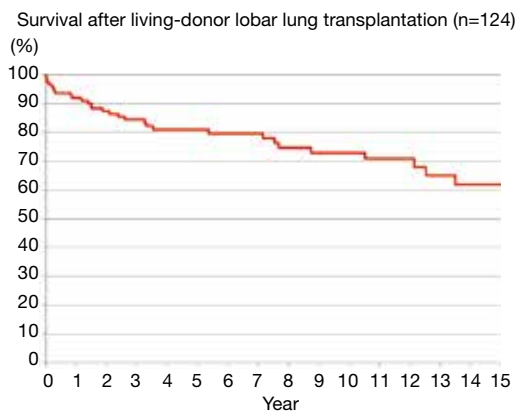
Survival after living-donor lobar lung transplantation (n=124)

Figure 6 Survival after living-donor lobar lung transplantation (n=124). The 5-, 10- and 15-year survivals were 80.8%, 72.6% and 61.7%, respectively.

Outcome of living-donors

Donors outcome is as important as that of the recipient. All our donors have returned to their previous life styles. However, long-term outcomes of donors have not been well documented because the donor follow-up is discontinued after 1 year.

Perioperative complications in living donors

According to previous reports, no perioperative death has been reported although relatively high morbidity after lobectomy has been described (29,30). Morbidity rates ranged from 20% to 60%. Report in 2006 from the Vancouver Forum Lung Group identified approximately 550 living lung donors (16). About 5% of live-donors have experienced complications requiring surgical or bronchoscopic intervention. Three technical differences between living donor lobectomy and standard lobectomy may explain the relatively high morbidity: (I) the pericardiotomy around the inferior pulmonary vein may lead to arrhythmias and pericarditis; (II) the division of the right lower lobe bronchus in an oblique fashion may increase the risk for bronchial fistula and stenosis; (III) heparin administration may result in perioperative bleeding.

We recently reported on our experience in donor lobectomy (31). Post-operative complications before discharge were seen in 20%. We also reported that health related quality of life and dyspnea of the living-donors deteriorated postoperatively in a prospective study (32).

Pulmonary function of living donors

As lung is not a regenerative organ, donors lose their pulmonary function permanently. We prospectively performed pulmonary function test 3, 6, and 12 months after donor lobectomy (33). Both FVC and forced expiratory volume in one second (FEV1) recovered up to about 90% of the preoperative value 1 year after donor lobectomy.

Outcome of LDLLT recipient

The USC group reported their long-term outcome on 123 LDLLT recipients including 39 children (13). Re-transplantation and mechanical ventilation were found to be risk factors for perioperative mortality. One-, 3-, and 5-year survival rate was 70%, 54%, and 45%, respectively. St. Louis group demonstrated similar results in 38 pediatric recipients receiving LDLLT (34).

As of June 2017, the author has performed 124 LDLLTs (47 at Okayama University and 77 at Kyoto University). There were 79 females and 45 males with ages ranging from 6 to 64 years (average 33.9 years). Twenty-nine of the patients were children and 95 were adults.

Recipient's diagnoses were listed in *Table 2*. Interstitial pneumonia, bronchiolitis obliterans, and pulmonary hypertension were the three major indications. All our patients were very sick and were depending on oxygen inhalation preoperatively. Seventy-five patients (61%) were bed bound and 14 (11%) were on a ventilator.

Bilateral LDLLT was performed in 108 patients and single LDLLT was performed in 16 small patients. There were 8 early deaths, for a hospital mortality of 6.5%. The causes of hospital death were graft failure due to excessive small grafts in 3, infection in 2, acute rejection in 1, heart failure in 1 and multi-organ failure in 1. There were 20 late deaths during a follow-up period of 2–225 months. The causes of late death were chronic lung allograft dysfunction (CLAD) in 8, malignancy in 7 including PTLD in 3, sepsis in 2, encephalitis in 1, and unknown cause in 2. The 5-, 10- and 15-year survivals were 80.8%, 72.6% and 61.7%, respectively (*Figure 6*).

Comparison with CLT

There are some advantages and disadvantages of LDLLT compared to CLT as summarized in *Table 3*. The graft ischemic time for LDLLT is significantly shorter than CLT.

Table 2 Diagnoses for living-donor lobar lung transplantation

Diagnoses	Number
Interstitial pneumonia	48
Bronchiolitis obliterans	35
Pulmonary hypertension	22
Bronchiectasis	6
Lymphangioleiomyomatosis	4
Re-transplantation	4
Cystic fibrosis	1
Emphysema	1
Others	3
Total	124

Table 3 Comparison between LDLLT and CLT

Variables	LDLLT	CLT
Waiting time	Short	Long
Schedule	Controllable	Uncontrollable
Ischemic time	Short	Long
Graft size	Small	Full
Primary graft failure	Infrequent	10–20%
Infection transmitted from graft	Infrequent	Frequent
Number of teams	3	2
Bronchial complication	Rare	5%
Chronic rejection	Often unilateral	Major cause of death

LDLLT, living-donor lobar lung transplantation; CLT, cadaveric lung transplantation.

Although relatively small grafts are implanted, primary graft failure seems to be less frequently encountered after LDLLT because of "small but perfect grafts".

The incidence of bronchial complications after CLT has been reported to be about 5%. Although we have accepted patients with high-dose systemic corticosteroid therapy in LDLLT, excellent bronchial healing was obtained in most of the recipients. Various factors including short donor bronchus, relatively high blood flow into the small grafts, normal lung parenchyma with short ischemic time, are contributors of better oxygen supply to the donor bronchus and excellent bronchial healing in LDLLT (35).

In our LDLLT experience at Kyoto University, airway complications developed in 4.7% and stenosis of the segmental bronchus was characteristic (36).

It is well known that bronchiolitis obliterans syndrome (BOS) is the major unsolved problem after CLT. We and the USC group reported less BOS incidence after LDLLT. It may be related to the shorter ischemic time in LDLLT. Interestingly, most of LDLLT recipients developed unilateral BOS and their FEV1 decline stopped within 9 months. Transplanting two different donor grafts appears to be a great benefit to the recipient because contra-lateral unaffected lung may function as a reservoir when BOS occurs unilaterally (37,38).

Acknowledgements

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Starnes VA, Lewiston NJ, Luikart H, et al. Current trends in lung transplantation. Lobar transplantation and expanded use of single lungs. J Thorac Cardiovasc Surg 1992;104:1060-5; discussion 1065-6.
2. Starnes VA, Barr ML, Cohen RG, et al. Living-donor lobar lung transplantation experience: intermediate results. J Thorac Cardiovasc Surg 1996;112:1284-90; discussion 1290-1.
3. Starnes VA, Barr ML, Schenkel FA, et al. Experience with living-donor lobar transplantation for indications other than cystic fibrosis. J Thorac Cardiovasc Surg 1997;114:917-21; discussion 921-2.
4. Date H, Aoe M, Nagahiro I, et al. Living-donor lobar lung transplantation for various lung diseases. J Thorac Cardiovasc Surg 2003;126:476-81.
5. Date H, Aoe M, Sano Y, et al. Improved survival after living-donor lobar lung transplantation. J Thorac Cardiovasc Surg 2004;128:933-40.
6. Date H, Tanimoto Y, Goto K, et al. A new treatment strategy for advanced idiopathic interstitial pneumonia: living-donor lobar lung transplantation. Chest 2005;128:1364-70.
7. Date H, Kusano KF, Matsubara H, et al. Living-

donor lobar lung transplantation for pulmonary arterial hypertension after failure of epoprostenol therapy. J Am Coll Cardiol 2007;50:523-7.

8. Mohite PN, Popov AF, Yacoub MH, et al. Live related donor lobar lung transplantation recipients surviving well over a decade: still an option in times of advanced donor management. J Cardiothorac Surg 2013;8:37.

9. Camargo SM, Camargo Jde J, Schio SM, et al. Complications related to lobectomy in living lobar lung transplant donors. J Bras Pneumol 2008;34:256-63.

10. Chen QK, Jiang GN, Ding JA, et al. First successful bilateral living-donor lobar lung transplantation in China. Chin Med J (Engl) 2010;123:1477-8.

11. Date H, Sato M, Aoyama A, et al. Living-donor lobar lung transplantation provides similar survival to cadaveric lung transplantation even for very ill patients†. Eur J Cardiothorac Surg 2015;47:967-72; discussion 972-3.

12. Kozower BD, Sweet SC, de la Morena M, et al. Living donor lobar grafts improve pediatric lung retransplantation survival. J Thorac Cardiovasc Surg 2006;131:1142-7.

13. Starnes VA, Bowdish ME, Woo MS, et al. A decade of living lobar lung transplantation: recipient outcomes. J Thorac Cardiovasc Surg 2004;127:114-22.

14. Date H. Current status and problems of lung transplantation in Japan. J Thorac Dis 2016;8:S631-6.

15. Chen F, Yamane M, Inoue M, et al. Less maintenance immunosuppression in lung transplantation following hematopoietic stem cell transplantation from the same living donor. Am J Transplant 2011;11:1509-16.

16. Barr ML, Belghiti J, Villamil FG, et al. A report of the Vancouver Forum on the care of the live organ donor: lung, liver, pancreas, and intestine data and medical guidelines. Transplantation 2006;81:1373-85.

17. Chen F, Fujinaga T, Shoji T, et al. Short-term outcome in living donors for lung transplantation: the role of preoperative computer tomographic evaluations of fissures and vascular anatomy. Transpl Int 2012;25:732-8.

18. Fujita T, Date H, Ueda K, et al. Experimental study on size matching in a canine living-donor lobar lung transplant model. J Thorac Cardiovasc Surg 2002;123:104-9.

19. Haddy SM, Bremner RM, Moore-Jefferies EW, et al. Hyperinflation resulting in hemodynamic collapse following living donor lobar transplantation. Anesthesiology 2002;97:1315-7.

20. Oto T, Date H, Ueda K, et al. Experimental study of oversized grafts in a canine living-donor lobar lung transplantation model. J Heart Lung Transplant 2001;20:1325-30.

21. Date H, Aoe M, Nagahiro I, et al. How to predict forced vital capacity after living-donor lobar-lung transplantation. J Heart Lung Transplant 2004;23:547-51.

22. Chen F, Kubo T, Yamada T, et al. Adaptation over a wide range of donor graft lung size discrepancies in living-donor lobar lung transplantation. Am J Transplant 2013;13:1336-42.

23. Chen F, Miwa S, Bando T, et al. Pulmonary arterioplasty for the remaining arterial stump of the donor and the arterial cuff of the donor graft in living-donor lobar lung transplantation. Eur J Cardiothorac Surg 2012;42:e138-9.

24. Kayawake H, Chen-Yoshikawa TF, Aoyama A, et al. Excellent outcome of donor lobectomy with various surgical techniques for the interlobar artery. Eur J Cardiothorac Surg 2017;51:279-83.

25. Date H, Shiraishi T, Sugimoto S, et al. Outcome of living-donor lobar lung transplantation using a single donor. J Thorac Cardiovasc Surg 2012;144:710-5.

26. Aoyama A, Chen F, Minakata K, et al. Sparing Native Upper Lobes in Living-Donor Lobar Lung Transplantation: Five Cases From a Single Center. Am J Transplant 2015;15:3202-7.

27. Chen F, Miyamoto E, Takemoto M, et al. Right and left inverted lobar lung transplantation. Am J Transplant 2015;15:1716-21.

28. Date H, Aoyama A, Hijiya K, et al. Outcomes of various transplant procedures (single, sparing, inverted) in living-donor lobar lung transplantation. J Thorac Cardiovasc Surg 2017;153:479-86.

29. Bowdish ME, Barr ML, Schenkel FA, et al. A decade of living lobar lung transplantation: perioperative complications after 253 donor lobectomies. Am J Transplant 2004;4:1283-8.

30. Yusen RD, Hong BA, Messersmith EE, et al. Morbidity and mortality of live lung donation: results from the RELIVE study. Am J Transplant 2014;14:1846-52.

31. Chen F, Yamada T, Sato M, et al. Postoperative pulmonary function and complications in living-donor lobectomy. J Heart Lung Transplant 2015;34:1089-94.

32. Chen F, Oga T, Sakai H, et al. A prospective study analyzing one-year multidimensional outcomes in living lung transplant donors. Am J Transplant 2013;13:3003-9.

33. Chen F, Fujinaga T, Shoji T, et al. Outcomes and pulmonary function in living lobar lung transplant donors. Transpl Int 2012;25:153-7.

34. Sweet SC. Pediatric living donor lobar lung transplantation. Pediatr Transplant 2006;10:861-8.

35. Toyooka S, Yamane M, Oto T, et al. Bronchial healing

after living-donor lobar lung transplantation. Surg Today 2009;39:938-43.

36. Miyamoto E, Chen-Yoshikawa TF, Higuchi H, et al. Stenosis of the segmental bronchus is a characteristic airway complication in living-donor lobar lung transplantation. J Heart Lung Transplant 2016;35:389-92.

37. Shinya T, Sato S, Kato K, et al. Assessment of mean transit time in the engrafted lung with 133Xe lung ventilation scintigraphy improves diagnosis of bronchiolitis obliterans syndrome in living-donor lobar lung transplant recipients. Ann Nucl Med 2008;22:31-9.

38. Miyamoto E, Chen F, Aoyama A, et al. Unilateral chronic lung allograft dysfunction is a characteristic of bilateral living-donor lobar lung transplantation. Eur J Cardiothorac Surg 2015;48:463-9.

Extracorporeal support, during and after lung transplantation: the history of an idea

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Abstract: During recent years, continuous technological innovation has provoked an increase of extracorporeal life support (ECLS) use for perioperative cardiopulmonary support in lung transplantation. Initial results were disappointing, due to ECLS-specific complications and high surgical risk of the supported patients. However, the combination of improved patient management, multidisciplinary team work and standardization of ECLS protocols has recently yielded excellent results in several case series from high-volume transplant centres. Therein, it was demonstrated that, although the prevalence of complications remains higher in supported patients, there may be no difference in long-term graft function between supported and non-supported patients. These results are important, because most of the patients who require ECLS support in lung transplantation are young and have no other chance to survive, but to be transplanted. Moreover, there is no device for "bridging to destination" therapy in lung transplantation. Of note, the evidence in favour of ECLS support in lung transplantation was never validated by randomized controlled trials, but by everyday experience at the patient bed-side. Here, we review the state-of-the-art ECLS evidence for intraoperative and postoperative cardiopulmonary support in lung transplantation.

Keywords: Extracorporeal life support (ECLS); lung transplantation; outcomes

Introduction

Experience is one of the cornerstones of the success of extracorporeal life support (ECLS) in lung transplantation (*Figure 1*). Over the last 20 years, from the initial case reports on ECLS as rescue before and after lung transplantation (1,2), experience and self-confidence with this technique has grown so much that it is impossible to think of lung transplantation without ECLS nowadays.

The integration of multidisciplinary team work (ECLS team) with the development of standardized management protocols and new technologies has yielded the good results of modern ECLS support (*Figure 1*), that have recently been published (3-15). Thus, ECLS use has broadened from pre-transplant bridging and post-transplant rescue therapy, to elective postoperative extension of intraoperative ECLS support in patients with idiopathic pulmonary arterial hypertension (16-19). While these indications have not been validated by randomized trials, the observational experience of each transplant center helped with refining the indications of ECLS.

Yet, the way was not paved only by successes. A more liberal use of ECLS support has been often criticized, because perioperative results were worse in patients supported by ECLS than in non-supported patients. While, arguably, these failures most likely were due to the
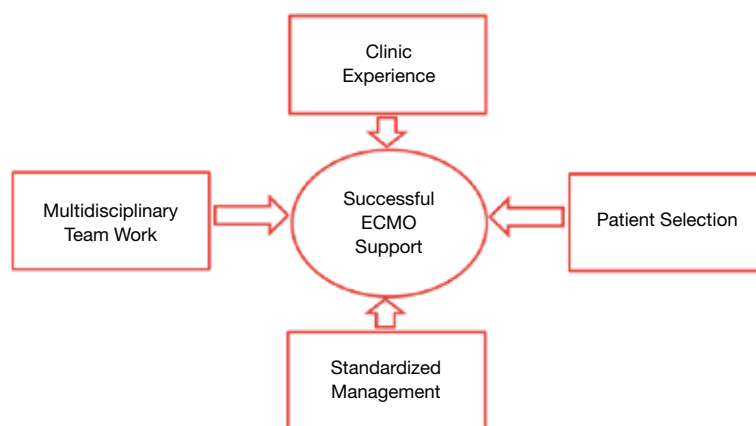
Figure 1 The cornerstones of a successful ECLS program. ECLS, extracorporeal life support.

underlying conditions of the patients, ECLS use is not void of complications, that often are provoked by the need of anticoagulation and arterial and venous vascular accesses. However, increasing expertise and refinements have steadily reduced the prevalence of such complications.

In this chapter, we report the current state-of the-art of ECLS support in patients undergoing lung transplantation. In particular, we focus on the intraoperative and post-transplant use of extracorporeal membrane oxygenation (ECMO), which is by far the most used ECLS support in lung transplantation, and hint briefly at the other support techniques and at ECMO use as a bridge to transplantation (BTT). A more thorough discussion on ECMO use as BTT has been recently reported elsewhere (19). At the end of this review, we present shortly our up-to-date experience with intraoperative and postoperative ECMO in lung transplantation.

ECLS: general considerations

Over recent years, ECLS technology has been steadily evolving, in order to cope with the requirements of less invasiveness, less prevalence of complications and of leaving supported patients awake and spontaneously breathing (20,21).

However, the basic setup of ECLS systems has remained practically unchanged. Since the inception of the Novalung (Novalung GmbH, Hechingen, Germany) polypentene fibre oxygenator in 2004 (20), the blood of the patient is usually drained by means of an outflow line to the ECLS system and is then pumped back to the patient through an inflow line, after oxygenation and decarboxylation. The

blood can be pumped by an external roller or centrifugal pump. Pumpless systems, driven by the pressure gradient between the femoral artery and vein, were en vogue for a number of years, but are less used nowadays, mostly for their inefficacy in oxygenation. A gas exchanger, usually a hollow-fibre oxygenator, is put in series and distally to the pump. Oxygenation depends on blood flow and FiO_2, and decarboxylation on sweep gas flow. There are no blood reservoirs and additional suction lines, as in the cardiopulmonary bypass (CPB) that is routinely used in open heart surgery. Therefore, ECLS systems are closed systems, sparing any air-blood interfaces and greatly reducing the surface area of the system, but they are particular susceptible to air embolism.

Figure 2 presents an overview of the ECLS systems that are used in lung transplantation. Different classifications have been proposed. ECLS can be used for pre-transplant, intraoperative or post-transplant support. In case of pre- and post-transplant support, ECLS may bridge patients to recovery or transplantation/re-transplantation. ECLS can support only the respiratory function (oxygenation/decarboxylation), or both the respiratory and cardio-circulatory functions. ECLS can be implanted using peripheral venous and arterial accesses, usually via the femoral vein and artery, or a central access, using the right atrium or the pulmonary artery for outflow and the aorta or left atrium for inflow (22).

ECMO: general considerations

ECMO is by far the most used ECLS system in lung transplantation. Other systems (20,22-27) such as the

Time	ECLS Type	Oxygenation	Decarboxylation	Cardiocirculatory support
Pretransplant	Peripheral veno-arterial ECMO	Peripheral organs	Yes	Yes
	Central veno-arterial ECMO	Central organs	Yes	Yes
	Veno-venous ECMO	Central organs	Yes	No
	Veno-venous-arterial ECMO	Central organs	Yes	Yes
	ECCO$_2$R	No	Partial	No
	Peripheral Novalung	No	Yes	No
	PA-LA Novalung	Yes	Yes	RV remodeling
	RA-PA Bypass	Yes	Yes	RV support
Intraoperative	Peripheral veno-arterial ECMO	Peripheral organs	Yes	Yes
	Central veno-arterial ECMO	Central organs	Yes	Yes
	Cardiopulmonary bypass (CPB)	Central organs	Yes	Yes
Post-transplant	Peripheral veno-arterial ECMO	Peripheral organs	Yes	Yes
	Central veno-arterial ECMO	Central organs	Yes	Yes
	Veno-venous ECMO	Central organs	Yes	No
	Veno-venous-arterial ECMO	Central organs	Yes	Yes

Figure 2 An overview of the available ECLS systems in lung transplantation. Cardiopulmonary bypass is also included. ECLS, extracorporeal life support.

extracorporeal carbon dioxide removal (ECOO$_2$R) and the peripheral or central Novalung (Novalung GmbH, Hechingen, Germany) have been less frequently used, and usually for bridging to transplantation. The pulmonary artery-left atrium (PA-LA) central Novalung requires a full sternotomy for implant. The peripheral Novalung requires cannulation of the femoral vessels and allows only decarboxylation. Both Novalung system depend on the cardiac function of the patient for pumping blood through the system. The ECOO$_2$R allows only a partial decarboxylation.

On the contrary, ECMO allows the complete spectrum of support in lung transplantation. Its versatility allows for pre-transplant support, which, in comparison with other ECLS systems, can be directly continued intraoperatively and post-transplant (*Figure 2*).

Veno-venous ECMO

The veno-venous ECMO modality allows only for respiratory support. A combination of veno-venous ECMO

and atrial septostomy may allow for cardiocirculatory support too, but its application has been limited by the difficulty to correctly size the septal defect, the tendency of the defect to shrink over time and the necessity to close the defect during ECMO weaning (20,28).

A dual or one site cannulation strategy can be used. In the case of a dual-site strategy, which is the preferred at our Institution, the outflow and inflow cannulas are usually placed percutaneously using the Seldinger technique in the femoral and internal jugular veins, respectively. This strategy allows for some degree of patient mobilisation but it does not allow for patient ambulation. Yet, the main advantage over the single cannula/double lumen strategy is the higher maximum ECMO blood flow, allowing for better oxygenation. In the case of one site cannulation strategy, a dual lumen cannula (Avalon Elite, Maquet, Rastatt, Germany) is placed percutaneously in the internal jugular vein or in the subclavian vein, under transoesophageal echocardiographic control. This strategy is more suitable, if an awake bridge to transplantation (BTT) strategy is planned, since it allows for patient ambulation. However,

cannula dislocation must be avoided during patient mobilisation. The veno-venous ECMO has been preferred for BTT and for bridging to recovery those patients who developed severe primary graft dysfunction (PGD) after transplantation (29). However, patients with lung fibrosis and secondary pulmonary arterial hypertension and transplanted patients with PGD and severe haemodynamic compromise may require peripheral or central veno-arterial ECMO (30).

Veno-arterial ECMO

Veno-arterial ECMO can be used for bridging patients with prevalent cardiocirculatory failure to lung transplantation, for intraoperative and post-transplant support. In this last case, intraoperative support can prophylactically be extended postoperatively in those patients with idiopathic pulmonary arterial hypertension, in order to avoid the development of severe cardiogenic oedema caused by diastolic dysfunction of the left ventricle (16-18). A peripheral or central cannulation strategy can be used.

In the case of peripheral cannulation, the femoral vein and artery are cannulated, usually percutaneously. In small size patients, such as children and small women, cut down and direct surgical exposition of the vessels are preferred. In babies, the jugular vein and a carotid artery are surgically isolated and cannulated. However, femoral veno-arterial ECMO guarantees oxygenation only of the periphery, because arterial blood flow from the ECMO at best reaches the distal aortic arch. Here, it mixes with blood pumped by the heart and oxygenated by the patient lungs, especially if the cardiac function is preserved. This effect usually does not cause any consequence in patients with idiopathic pulmonary arterial hypertension, where the oxygenatory function of the lung is preserved. On the contrary, if lung function is impaired, as in patients with pulmonary fibrosis, central organs such as the heart and brain are perfused with poorly oxygenated blood (watershed effect, Harlequin Syndrome). Controlling the arterial blood gases from the right radial artery and continuously measuring the peripheral oxygen saturation from the right hand or ear lobes are useful tools for recognizing the development of such syndrome.

Centrally cannulated veno-arterial ECMO might mitigate this problem, since arterial blood can be directly infused into the ascending aorta (31). However, it requires a full sternotomy and hinders patient mobilization and an awake BTT strategy, not the least because additional major surgery is necessary for ECMO explant. Alternatively,

the arterial inflow cannula can be placed into the right subclavian artery, through the interposition of a Dacron prosthesis. This configuration ("sport" configuration) allows for patient ambulation and rehabilitation (4,20). Another option is to create a veno-veno-arterial ECMO circuit by adding an arterial inflow cannula into the jugular vein (32), in parallel with the arterial inflow into the femoral artery. In this case, however, particular care must be paid in balancing the flows through the two inflow cannulas, according to the respiratory and cardiocirculatory needs of the patient. As a rule of thumb, 1/3 of the inflow should be dedicated for respiratory support and the remaining for cardiocirculatory support. The use of a flowmeter is essential if this support modality is used.

Careful attention must be paid to the development of ischemia in the leg, where the arterial inflow cannula is placed, especially in BTT patients and in patients with post-transplant veno-arterial ECMO (22,33,34). At our institution, an antegrade leg perfusion cannula is placed distally to the inflow arterial cannula, whenever possible. Moreover, the arterial pulses as well as cannula patency are checked regularly. Near-infrared spectroscopy is used to continuously monitor the oxygen saturation of the leg, where the inflow cannula has been placed.

ECMO implant technique and management

For each ECMO modality, cannula sizes depend on patient size and blood flow requirements. Cannulation strategy and choice of cannula types depend on institutional preference. Cannula insertion and placement can be guided by sonography and controlled by transoesophageal echocardiography, especially in the case of the dual lumen cannula for veno-venous ECMO. However, these tools are not always available, especially in the emergency setting. At our institution, a chest X-ray is performed to control cannula position after ECMO implant.

The management of anticoagulation at ECMO implant and during ECMO support is of paramount importance to avoid bleeding. Many Institutions have developed their own protocols. At our Institution, we usually infuse a bolus of 5.000 units of unfractionated heparin before cannula insertion and then guide anticoagulation by regularly measuring the activated clotting time (ACT) every 4 hours, as well as the international normalized ratio (INR), the Quick value, the activated partial thromboplastin time (aPTT), the plasma levels of fibrinogen, factor V and II at least twice daily. Heparin is started usually not before

Table 1 Published case series on ECMO as bridge to transplantation

Study	N patients	Patients bridged to transplant	Intention-to-treat survival	Survival after transplantation
Bermudez et al., 2011 (35)	17	15 (88.2)	–	65% at 3 years
Fuehner et al., 2012 (36)	26	20 (76.9)	–	80% at 6 months
Lang et al., 2012 (37)	38	34 (89.5)	–	60% at 1 year
Javidfar et al., 2012 (38)	18	13 (72.2)	61% at 3 months	–
Toyoda et al., 2013 (39)	31	24 (77.4)	–	74% at 2 years
Lafarge et al., 2013 (40)	36	30 (83.3)	50.4% at 2-years	60.5% at 2 years
Dellgren et al., 2015 (41)	20	16 (80.0)	62% at 1 year	69% at 1 year
Yeo et al., 2017 (42)	19	14 (73.7)	57.9%	64.3%
Biscotti et al., 2017 (4)	72	40 (55.6)	37.5% at 2 years	84% at 2 years
Todd et al., 2017 (3)	–	12	–	100% at 1 year
Ius et al., 2018 (5)	87	68 (78.2)	52% at 5 years	71% at 5 years
Hoetzenecker et al., 2018 (6)	71	63 (88.7)	51% at 5 years	60% at 5 years
Hakim et al., 2018 (7)	30	26 (86.7)	70% at 3 years	80% at 3 years

Values are reported as n (%) or %.

48 hours after ECMO implant, aiming at an ACT set at 160–180 s.

The management of ECMO patient before or after lung transplantation will be further discussed in the following paragraphs and it depends on the patient cardiopulmonary conditions and the type of support. Anyway, at our institution, heart rate, central venous pressure, arterial blood pressure, pulmonary arterial pressure, body temperature, and peripheral oxygen saturation are continuously monitored. Antibiotic therapy is empirically performed with flucloxacillin, meropenem and voriconazole, or antibiogram-guided, if there is evidence of any pathogen.

ECMO as a bridge to transplantation

Table 1 reports the most recent case series on ECMO as BTT. These case series show that survival results have been steadily improving over the last years and that (I) a careful patient selection, (II) the implementation of awake ECMO protocols and (III) center transplant volume and experience were fundamental to this improvement (3-7,35-49).

Patient selection implies ethical issues, because, due to the organ donor shortage, it could be questioned whether organs should be really offered to such high-risk recipients. However, patients selected for undergoing BTT often are younger than other wait list patients. In contrast to patients listed for a heart transplantation, there is no device available for bridge to destination therapy in lung transplantation. Moreover, the most recent case series have even shown that graft survival was similar in patients who were bridged to transplantation with ECMO and those who were not. Todd *et al.* reported a 1-year survival of 100% in the bridge to transplant group and of 91% in the non-bridge to transplant group, with an excellent functional status in both groups (3). Our group has recently shown that overall graft survival did not differ between BTT and non-BTT patients (79% *vs.* 90% and 61% *vs.* 68% at 1 and 5 years, respectively, P=0.13) and that ECMO as BTT did not emerge as risk factor for graft survival at the multivariate analysis (5).

Anyway, each institution has its protocol for selecting candidates for BTT, whose indications and contraindications have been recently summarized by Loor *et al.* (19). At our institution, we usually avoid considering BTT for patients who showed irreversible end-organ damage or sepsis. Older age remains a relative contraindication. For example, we usually do not proceed to transplanting older patients with pulmonary fibrosis if they require invasive mechanical ventilation while on ECMO. Patients requiring retransplantation are also carefully selected, since results of ECMO as BTT in these

patients have been less satisfactory (6,45). Moreover, we prefer considering for ECMO as BTT those patients who have already been listed at our institution. Patients without previous transplant evaluation who were transferred to our intensive care unit from a peripheral hospital under mechanical ventilation and ECMO support are considered for transplant only after extubation and careful evaluation of end-organ damage (5).

The introduction of the "awake" strategy as early as 2008 represented an important milestone in the management of pre-transplant patients at our institution (36). Since then, many other case series have validated the benefit of spontaneous breathing and mobilisation during ECMO support, not only before, but also after lung transplantation (3-6,16,17,44,45,48). Moreover, new cannulas and implant techniques have dramatically improved the chance of patients being rehabilitated during ECMO support (50-53). Thereby, the patient muscular deconditioning due to the immobilisation and mechanical ventilation are remarkably reduced. Hayanga *et al.* have recently demonstrated that extubated patients on ECMO as BTT fare better than intubated patients on ECMO including those patients who required only mechanical ventilation before transplantation (54).

ECMO for intraoperative support

In 2010, we changed our protocol for intraoperative cardiopulmonary support during lung transplantation, replacing CPB with peripheral veno-arterial ECMO (55). Our decision was based on the following considerations: (I) the versatility of intraoperative ECMO, which allowed for continuing support in those patients who could not be weaned from it; (II) the possibility of performing major cardiac surgery, for example coronary artery bypass grafting (CABG) on beating heart technique with ECMO support; (III) the lower amount of heparin required by ECMO in comparison to CPB; (IV) the lower amount of priming volume in ECMO circuits; (V) the lower degree of systemic inflammatory reaction (SIRS) after ECMO; and (VI) the feasibility of intraoperative ECMO support as previously evidenced by the colleagues from Vienna, Austria (56).

We do not exclude a priori the use of CPB and still use it in the following situations: (I) concomitant need of repairing an intracardiac defect, such as an atrial septal defect, and (II) in case of unexpected massive blood loss, for example when the pulmonary artery is accidentally injured during insolation for clamping. Regarding the first point,

we perform the intracardiac repair under CPB, wean it and then proceed to lung transplantation without CPB support.

In a recent publication (12), we have divided patients undergoing intraoperative ECMO support in two categories, those requiring an a-priori ECMO support and those without a-priori indication for ECMO. A priori ECMO was reserved for the following patients: (I) patients already bearing ECMO as BTT; (II) patients with idiopathic pulmonary arterial hypertension as indication to transplantation or secondary supra-systemic pulmonary hypertension; (III) patients where a lobar transplantation was planned in order to avoid hyper-perfusion of the transplanted lobe during single lung ventilation; and (IV) patients requiring concomitant CABG. In these patients, it would not be possible to perform transplantation safely without ECMO support. Strikingly, we demonstrated that survival did not differ between patients who required intraoperative ECMO and those who did not, and that the intraoperative use of ECMO did not emerge as a risk factor for in-hospital mortality or mortality after hospital discharge (12).

Since 2010, several case series and meta-analyses have demonstrated the superiority of ECMO *vs.* CPB for intraoperative support and better outcomes in lung transplantation (*Table 2*) (8-11,13,14,57-60). Some authors have recently proposed the routine use of intraoperative ECMO in lung transplantation, in order to allow controlled perfusion and protective ventilation of the graft during transplantation and thus reduce the risk of later PGD (14,57).

These suggestions should be regarded with caution, however, given that ECMO therapy is associated with ECMO-specific complications, such as bleeding and vascular complications (33,34,61), which might impair perioperative results. Central cannulation of the aorta and right atrium has been proposed as a strategy to reduce vascular complications (31). However, central cannulation often requires a clamshell incision and, in those patients requiring post-transplant continued ECMO support, later switch to peripheral ECMO.

In our opinion, the identification of those patients who really need ECMO support and the avoiding of ECMO implant under urgent/emergent conditions, for example during or after pneumonectomy, may help with reducing ECMO-specific complications. Therefore, we have previously designed a decision algorithm that helps with identifying those patients who are at risk of requiring intraoperative ECMO support (12). In particular, before starting the operation, it is important to check the

Table 2 Published case series on ECMO for intraoperative support during lung transplantation

Study	N patients	Survival	Rethoracotomy for bleeding	Vascular complications
Biscotti *et al.*, 2014 (9)	47 (14.9)	94% at 30 days	3 (6.4)	3 (6.4)
Bermudez *et al.*, 2014 (8)	49 (7.6)	81% at 1 year	4 (8.2)	
Machuca *et al.*, 2015 (10)	33	94% at 3 months	3 (9.1)	
Hoechter *et al.*, 2015 (11)	27 (14.4)	82% at 1 year	11 (40.7)	
Ius *et al.*, 2016* (12)	170 (28.6)	68% at 4 years	29 (17.1)	10 (5.9)
Yu *et al.*, 2016 (57)	41	63% at 1 year	12 (29.3)	0
Cosgun *et al.*, 2017 (58)	134 (46.0)	53% at 5 years		
Glorion *et al.*, 2018[§] (31)	103 (49.0)		22 (21.4)	26 (25.2)
Pettenuzzo *et al.*, 2018 (13)	15 (28.8)	87% at 6 months		
Hoetzenecker *et al.*, 2018[†] (14)	466 (80.0)	74% at 5 years	41 (8.8)	11 (2.4)

Values are reported as n (%). *, 95 patients with a priori ECMO implant, 75 patients without a priori ECMO implant. [§], 49 patients with peripheral veno-arterial ECMO and 54 patients with central veno-arterial ECMO. [†], 343 patients with intraoperative ECMO support only, 123 patients with prolonged postoperative ECMO support.

echocardiographic reports, the results of the right heart catheterization and the X-ray of the recipient. The presence of a secondary pulmonary arterial hypertension, of a dilated and hypertrophied right ventricle, and of small thoracic cavities at the chest X-ray should alert the surgeon to the higher risk of requiring intraoperative ECMO. Close communication with the retrieval surgeon is of paramount importance. The presence of contusions or chronic atelectasis in one of the lungs should prompt the decision of implanting the better lung first. Before incision, the surgeon should check that nitric oxide (NO) ventilation is switched on. During implantation, the surgeon should closely communicate with the anaesthesiologist, in order to identify possible signs of respiratory and haemodynamic instability and counteract against them quickly. If cardiopulmonary conditions worsen after test clamping of the right or left pulmonary arteries, the surgeon should look for potentially reversible causes, and to proceed with implantation of an ECMO if cardiopulmonary instability persists. Usually, indication for ECMO implant is set if a combination of the following conditions ensues: (I) hypercapnia; (II) decrease of arterial saturation less than 90%; (III) cardiac index less than 2 L/min/m^2; and (IV) increase of the pulmonary arterial pressure to supra-systemic values. The second evaluation is performed after implanting the first lung, at clamping of second pulmonary artery, checking whether the freshly implanted lung is able to yield adequate oxygenation

and accommodate the entire cardiac output (12). In patients at risk, we pre-emptively place guidewires in the right femoral vein and artery to prepare a safe and quick ECMO implant if necessary later.

At the end of transplantation, before closing the thoracotomies, the possibility of explanting the ECMO is evaluated by checking the arterial blood gases and pulmonary arterial pressure at 100% FiO$_2$. If these parameters are satisfactory, ECMO is explanted. In patients with idiopathic pulmonary arterial hypertension, veno-arterial ECMO might be left in place as per institutional protocol. Careful attention is paid to meticulous haemostasis. We suggest antagonizing heparin with half-dose protamine. Recently, Narm *et al.* have shown that increasing donor age, donor PaO$_2$ and increasing operation duration were independent risk factors of weaning failure from intraoperative ECMO in their experience (62).

A particular mention deserves the possibility of performing concomitant CABG and lung transplantation, as a beating heart technique and under ECMO support. Indeed, it is not always possible to treat the coronary artery disease (CAD) with PTCA and stenting before transplantation, especially if a complex stenosis or a two-vessel CAD are present. At our institution, we perform lung transplantation and CABG using a clamshell incision. We usually perform first the lung transplantation and then the CABG. ECMO can be implanted at the beginning of

Table 3 Published case series on secondary ECMO implant after lung transplantation

Study	N patients	Survival	Weaned patients	Time ECMO-weaning (days)
Meyers *et al.*, 2000 (64)	12 (2.7)	–	8 (66.6)	4.2 (mean)
Dahlberg *et al.*, 2004 (65)	16 (9.3)	46% at 2 years	–	–
Oto *et al.*, 2004 (66)	10 (2.1)	–	4 (40.0)	4 (mean)
Mason *et al.*, 2006 (67)	22 (4.0)	41% at 1 year[†]	–	4 (median)
Fischer *et al.*, 2007 (68)	151	42% at hospital discharge	–	6 (mean)
Bermudez *et al.*, 2009 (69)	58 (7.6)	40% at 1 year	39 (67.2)	5.5 (mean)
Hartwig *et al.*, 2012 (15)	28 (6.0)	64% at 1 year	27 (96.4)	3.6 (mean)
Marasco *et al.*, 2012 (70)	24	25% at hospital discharge	14 (58.3)	4.5 (median)
Mulvihill *et al.*, 2018 (71)	107 (5.1)	62% at 6 months	–	–

Values are reported as n (%). [†], survival was 41% at 1 year in patients with early graft failure and acute rejection and only 3% in patients with sepsis and pneumonia.

transplantation or at its end, before CABG. Since both the internal mammary arteries are sacrificed during the clamshell incision, saphenous vein grafts are usually used. The Octopus system (Medtronic Inc, Minn., MN, USA) might be used for stabilizing the coronary artery during anastomosis. ECMO is usually explanted at the end of CABG.

ECMO for postoperative support

The development of severe graft dysfunction after lung transplantation is a dramatic event and it sometimes requires secondary ECMO therapy for graft rescue, if conservative therapy fails. Graft function and survival were worse in patients who required secondary ECMO than in patients who did not require it (15,63-71) (*Table 3*). However, early recognition of incoming graft dysfunction with prompt ECMO implant has partially improved the prognosis of these complications (15).

Severe graft dysfunction is mainly due to the development of primary graft dysfunction early after transplantation, and due to acute rejection or pneumonia later after transplantation. Mason *et al.* (67) and Marasco *et al.* (70) have shown that secondary ECMO for early causes of graft dysfunction yielded better weaning and survival results than secondary ECMO for later causes. This finding shows that ECMO therapy for severe PGD should not be limited to a mere rescuing role but should be considered pre-emptively in those patients who are at particular higher risk of developing it (72,73), such as those transplanted for idiopathic pulmonary arterial hypertension.

In these patients, PGD was supposed to be a consequence of endothelial injury from shear-stress forces applied by a well-trained right ventricle resulting in subsequent pulmonary oedema. However, we and others have recently demonstrated that the primary mechanism causing PGD might be not right ventricle-related, but more likely due to diastolic dysfunction of the left ventricle, and should rather be called cardiogenic oedema. In fact, the long-standing underfilling of the left ventricle in the presence of reduced cardiac output secondary to very high pulmonary vascular resistance may result in deconditioning of the left ventricle, rendering the left ventricle incapable of handling a normal preload in the early postoperative period (16). The characteristic pulmonary oedema of PGD is a consequence of a sudden increase of the left ventricular end diastolic pressure and of the left atrial pressures soon after graft reperfusion, aggravated further after extubation of the patient. The postoperative use of veno-arterial ECMO provides time for gradual adaption of the left ventricle to the new haemodynamic situation after transplantation. The importance of the left ventricular diastolic dysfunction in causing PGD has been recently demonstrated by Porteous *et al.* (74). They suggested that veno-arterial ECMO may allow for controlled filling and recovery of the left ventricle, preventing acute increases in pulmonary venous pressure in the early period after lung transplantation.

Therefore, at our institution, patients with idiopathic pulmonary arterial hypertension are usually transplanted under veno-arterial ECMO support, which is not weaned at the end of transplantation, but continued directly

in the postoperative period. Full ECMO support is maintained for at least 5 days. In the meanwhile, patients are extubated (awake ECMO) and undergo rehabilitation, whenever possible. Cardiac function is regularly checked with transthoracic echocardiography. Pulmonary arterial and left atrial pressures are continuously monitored using a pulmonary arterial catheter and an additional pressure line, that was previously placed in the left atrium during transplantation, respectively. Beyond day 5 after transplantation, ECMO weaning is initiated while controlling for left ventricular function during intermittent reduction of ECMO blood flow to 0.6 L/m^2 body surface area. When there are neither echocardiographic signs of left ventricular diastolic dysfunction nor increases of pressure in left atrium over 10 mmHg during reduction of blood flow, the ECMO flow is reduced in 0.5 L per minute steps, until a flow of 0.6 L/m^2 body surface area is reached and then the ECMO is explanted (16). During ECMO reduction, a negative fluid balance is carefully maintained. Moreover, we keep the arterial blood pressure under a mean of 80 mmHg using beta-blockers, which are usually continued after ECMO explant, and avoid the use of any inotropic drugs, since left and right systolic ventricular function is usually preserved in these patients. We have recently published our complete surgical experience in patients with severe pulmonary arterial hypertension treated with this ECMO protocol (17). We showed that survival did not differ between patients transplanted for severe pulmonary hypertension and those transplanted for other underlying conditions and that a normal left ventricular diastolic function was achieved at last echocardiographic control. In patients with severe pulmonary hypertension, 5-year survival was 70%, which was comparable with the 5-year survival of patients transplanted for other conditions (69%).

Excellent results using prolonged veno-arterial ECMO support in patients with idiopathic pulmonary arterial hypertension have been recently published by the Vienna group (18). They started using veno-arterial ECMO for idiopathic pulmonary arterial hypertension as early as 2000 and reported a 5-year survival of 87.4%. Yet, the management protocol is different. Intraoperatively, they used centrally implanted veno-arterial ECMO, which was switched to peripherally implanted ECMO at the end of transplantation. They kept patients on veno-arterial ECMO support for a shorter period of time (median of 2.5 days) after transplantation and did not proceed to extubation before ECMO weaning. After ECMO weaning, they prefer ventilating patients with a protective low tidal

volume pattern and address aggressively the normalization of fluid balance, which explains the higher prevalence of hemodialysis treatment in their experience.

Considering the Hannover and Vienna experience together, we suggest that patients with severe pulmonary arterial hypertension who were under prophylactic postoperative veno-arterial ECMO support should not be considered anymore as having automatically PGD 3 scores, as reported by the most recent consensus statement on PGD (72).

Finally, although other recipient and donor risk factors for PGD have been reported (73), we apply our ECMO protocol only in patients with severe pulmonary hypertension. We carefully monitor the other patients, in order to detect the first signs of PGD as soon as possible.

Hannover experience with intraoperative ECMO

Before concluding, we present briefly our recent experience with ECMO in lung transplantation. Results are reported in *Tables 4*-7, and in *Figures 3*,*4*. Data were reported as n (%) and median (interquartile range, IQR), for categorical and continuous variables, respectively.

Between January 2010 and May 2018, 1,042 patients underwent lung transplantation at our institution. Among these patients, 22 (2%) patients were transplanted with CPB support and were thus excluded from the analysis.

Among the remaining 1,020 (98%) included patients, 281 (28%) required intraoperative veno-venous or veno-arterial ECMO support, and 739 (72%) patients did not. Follow-up ended in June 2018, was 100% complete and amounted to a median of 37 (IQR, 17–61) months.

ECMO patients showed a higher surgical risk than no ECMO patients, with a higher prevalence of pulmonary fibrosis and idiopathic pulmonary arterial hypertension as indication to transplantation. ECMO patients were younger than no ECMO patients and were more often females. Of note, donor characteristics, except for female sex, did not differ between ECMO and no ECMO patients.

Postoperatively, prevalence of major complications was higher in ECMO than no ECMO patients, whose consequence was a higher in-hospital mortality in ECMO than no ECMO patients. Although overall survival was worse in ECMO patients, survival did not substantially differ among groups at 5 years (*Table 7*, *Figure 4*). Moreover, survival conditioned to hospital discharge and freedom from chronic lung allograft dysfunction (CLAD) did not differ between groups over the whole study period.

Table 4 Preoperative recipient data in patients who required ECMO support during transplantation and in patients who did not

Variable	No intraoperative ECMO (n=739)	Intraoperative ECMO (n=281)	P
Female sex	331 (44.8)	155 (55.2)	0.003
Age (years)	53 [40–59]	49 [31–57]	<0.001
Age <18 years	37 (5.0)	33 (11.7)	<0.001
Age >60 years	110 (14.9)	39 (13.9)	0.68
BSA (m^2)	1.8 [1.6–1.9]	1.7 [1.5–1.9]	0.061
Coronary artery disease	54 (7.3)	28 (10.0)	0.16
Blood group			
A	339 (45.9)	129 (45.9)	0.99
B	82 (11.1)	28(10.0)	0.60
AB	35 (4.7)	11 (3.9)	0.57
0	282 (38.2)	113 (40.2)	0.55
CMV risk			
Low	162 (21.9)	56 (19.9)	0.48
Intermediate	337 (45.6)	115 (40.9)	0.18
High	239 (32.3)	110 (39.1)	0.041
Transplant indication			
COPD	269 (36.4)	12 (4.3)	<0.001
Pulmonary fibrosis	210 (28.4)	122 (43.4)	<0.001
Cystic fibrosis	167 (22.6)	45 (16.0)	0.021
Pulmonary hypertension	0 (0)	63 (22.4)	<0.001
Re-transplant	51 (6.9)	17 (6.0)	0.63
Other	42 (5.7)	22 (7.8)	0.21
Associated pulmonary artery hypertension	230 (31.1)	182 (64.8)	<0.001
LAS score	35.1 [32.9–39.2]	42.7 [35.9–63.5]	<0.001
Preoperative mechanical ventilation	6 (0.8)	25 (8.9)	<0.001
Preoperative intensive care unit	22 (3.0)	84 (29.9)	<0.001
Preoperative ECMO/iLA	0 (0)	74 (26.3)	<0.001
iLA		1 (1.4)	
VA ECMO		29 (39.2)	
VV ECMO		47 (63.5)	

Values are expressed as median [IQR, interquartile range] or N of patients (%). BSA, body surface area; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; iLA, interventional Lung Assist Novalung; LAS, lung allocating score; VA, veno-arterial; VV, veno-venous.

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Table 5 Donor and intraoperative recipient characteristics in patients who required ECMO support during transplantation and in patients who did not

Variable	No intraoperative ECMO (n=739)	Intraoperative ECMO (n=281)	P
Donor characteristics			
Female sex	353 (47.8)	166 (59.1)	0.001
Age (years)	48 [35–57]	47 [35–58]	0.98
Age >70 years	34 (4.6)	19 (6.8)	0.15
BSA (m^2)	1.9 (1.8–2.0)	1.9 (1.7–2.0)	0.031
Ventilation time (days)	4 [2–7]	4 [2–7]	0.54
pO$_2$ (100%, mmHg)	387 [316–451]	395 [329–450]	0.79
Smoking history	312 (42.2)	117 (41.6)	0.91
Contusion	70 (9.5)	28 (10.0)	0.79
Aspiration	39 (5.3)	17 (6.0)	0.62
Lung preservation			
Celsior	575 (77.8)	231 (82.2)	0.15
Portable EVLP	48 (6.5)	18 (6.4)	0.96
Intraoperative recipient characteristics			
Thoracotomy			
Sternum sparing	721 (97.6)	242 (86.1)	<0.001
Clamshell	18 (2.4)	39 (13.9)	<0.001
Type of transplantation			
Single lung	10 (1.4)	12 (4.3)	0.004
Double lung	729 (98.6)	269 (95.7)	0.004
Intraoperative ECMO			
A priori	–	169 (60.1)	–
Not planned	–	112 (39.9)	–
Postoperative extended ECMO		92 (32.7)	–
VA ECMO	–	91 (98.9)	–
VV ECMO	–	1 (1.1)	–
Ischemic time (min)			
First lung	407 [322–513]	419 [337–502]	0.36
Second lung	520 [436–626]	550 [453–630]	0.038
Lung volume reduction			
Atypical	7 (0.9)	14 (5.0)	<0.001
Lobar	10 (1.4)	32 (11.4)	<0.001

Values are expressed as median [IQR, interquartile range] or N of patients (%). BSA, body surface area; ECMO, extracorporeal membrane oxygenation; EVLP, ex-vivo lung perfusion; VA, veno-arterial; VV, veno-venous.

Table 6 Postoperative data in patients who required ECMO support during transplantation and in patients who did not

Variable	No intraoperative ECMO (n=739)	Intraoperative ECMO (n=281)	P
PGD score grade 2 or 3			
24 hours	72 (9.7)	88 (31.3)	<0.001
48 hours	66 (8.9)	99 (35.2)	<0.001
72 hours	47 (6.4)	81 (28.8)	<0.001
Rethoracotomy for bleeding	30 (4.1)	50 (17.8)	<0.001
New dialysis	27 (3.7)	56 (19.9)	<0.001
Atrial fibrillation	69 (9.3)	50 (17.8)	<0.001
Vascular complications	2 (0.3)	27 (9.6)	<0.001
Cerebrovascular disorder	10 (1.4)	5 (1.8)	0.57
Postoperative pulsed steroid therapy	185 (25.0)	107 (38.1)	<0.001
Blood products, overall			
PRBCs (units)	5 [3–8]	12 [7–26]	<0.001
PC (units)	0 [0–2]	2 [2–7]	<0.001
FFP (units)	4 [3–6]	9 [5–16]	<0.001
Secondary ECMO	5 (0.7)	15 (5.3)	<0.001
VA ECMO	4 (0.5)	11 (3.9)	
VV ECMO	1 (0.1)	5 (1.8)	
Tracheostomy	45 (6.1)	72 (25.6)	<0.001
Ventilation time, hours	1 [1–1]	2 [1–4]	<0.001
ICU stay, days	2 [1–3]	7 [3–19]	<0.001
Hospital stay, days	23 [21–26]	28 [22–49]	<0.001
In-hospital mortality	19 (2.6)	34 (12.1)	<0.001
Immunosuppressive therapy at discharge after transplantation[†]			
Cyclosporine	202 (28.1)	68 (27.5)	0.87
Tacrolimus	518 (71.9)	179 (72.5)	0.87
Immunosuppressive therapy at last outpatient control[†]			
Cyclosporine	159 (22.1)	45 (18.2)	0.19
Tacrolimus	555 (77.1)	198 (80.2)	0.31

Values are expressed as median [IQR, interquartile range] or N of patients (%). [†], in-hospital deaths (n=53) are excluded. FFP, fresh frozen plasma; ICU, intensive care unit; PC, platelet concentrate; PGD, primary graft dysfunction; PRBCs, packed red blood cells; VA, veno-arterial; VV, veno-venous.

Table 7 Outcomes in patients who required ECMO support during transplantation and in patients who did not

Variable	No intraoperative ECMO (n=739)	Intraoperative ECMO (n=281)	P
Patient survival, overall (%)			0.020
3 years	84±2	74±3	
5 years	72±2	68±3	
Patient conditioned to hospital discharge (%)			0.50
3 years	86±2	85±3	
5 years	74±2	78±3	
Graft survival (%)			0.031
3 years	82±2	73±3	
5 years	69±2	64±3	
Causes of death after hospital discharge[†]			
CLAD	72 (10.0)	22 (8.9)	0.62
Infection	20 (2.8)	9 (3.6)	0.49
Malignancy	17 (2.4)	4 (1.6)	0.49
Cardiac	13 (1.8)	3 (1.2)	0.77
Other	14 (1.9)	6 (2.4)	0.64
Freedom from biopsy-confirmed rejection (%)			0.71
1 year	65±2	61±4	
3 years	56±2	52±4	
5 years	52±2	51±4	
ISHLT biopsy grade*			
A1	227 (34.5)	80 (40.2)	0.14
A2	82 (12.5)	23 (11.6)	0.73
A3	4 (0.6)	1 (0.5)	0.86
Freedom from pulsed steroid therapy (%)			0.90
1 year	54±2	55±3	
3 years	41±2	39±3	
5 years	37±2	34±4	
Freedom from CLAD (%)			0.98
3 years	77±2	75±3	
5 years	67±2	66±4	

Values are expressed as mean ± SD (%) or N of patients (%). [†], patients who died before hospital discharge (n=53) were not considered, Intraoperative ECMO n=247, No intraoperative ECMO n=720. *, No intraoperative ECMO n=658, Intraoperative ECMO n=199. CLAD, chronic lung allograft dysfunction; ISHLT, International Society of Heart and Lung Transplantation.
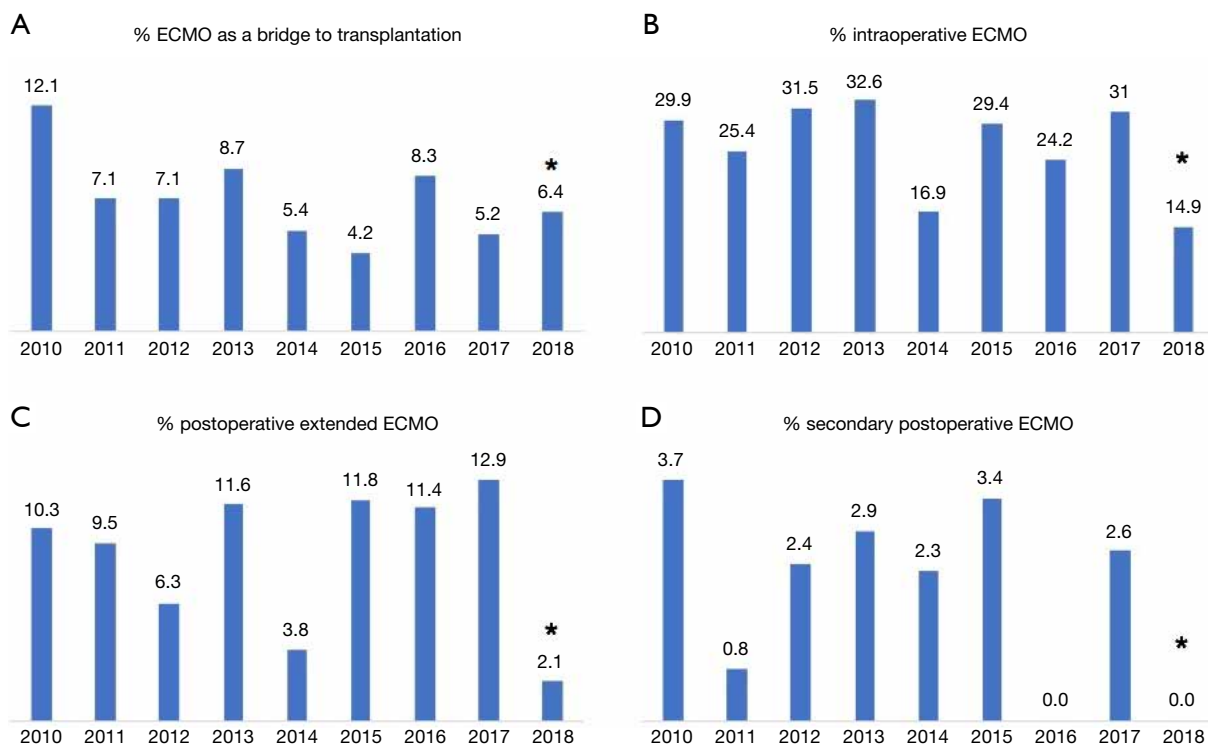
Figure 3 The figure shows the percentage of: (A) patients bridged to lung transplantation with ECMO per each study year at our institution; (B) patients requiring intraoperative ECMO support; (C) patients requiring extended postoperative ECMO support; and (D) patients requiring secondary ECMO support after transplantation. At our institution, 107 patients were transplanted in 2010, 126 in 2011, 127 in 2012, 138 in 2013, 130 in 2014, 119 in 2015, 132 in 2016, 116 in 2017, and 47 until May 2018.
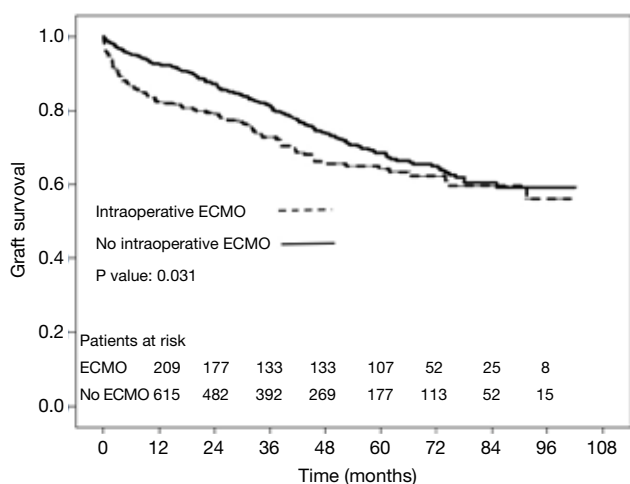
Figure 4 The graft survival (intended as freedom from mortality and retransplantation) in patients who required ECMO support during lung transplantation *vs.* patients who did not require it, at our institution. Patients at risk are reported above the X axis.

On the contrary, median values of forced expiratory volume in 1 second (FEV1, % predicted) at 1 year and at last control (performed at median of 35 months after transplantation) were significantly lower in ECMO than in no ECMO patients (75 *vs.* 90, P<0.001; and 66 *vs.* 77, P<0.001, respectively). However, this finding can be also explained by the higher prevalence of females in the ECMO group.

Conclusions

The introduction of ECLS for cardiopulmonary support in lung transplantation has revolutionized the management of transplanted patients. It has allowed for an expansion of the transplant indications, and has allowed for transplanting patients who were inexorably condemned to a certain death before its introduction.

This revolution was not validated by randomized

controlled trials, but by the everyday experience at the patient bed-side.

Further progress in ambulation of ECMO patients, in the reduction of complications, and eventually even in full implantability of the devices are expected and will revolutionize the field of lung transplantation for the years to come.

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Footnote

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References

1. Jurmann MJ, Haverich A, Demertzis S, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation. Eur J Cardiothorac Surg 1991;5:94-7.
2. Demertzis S, Haverich A, Ziemer G, et al. Successful lung transplantation for posttraumatic adult respiratory distress syndrome after extracorporeal membrane oxygenation support. J Heart Lung Transplant 1992;11:1005-7.
3. Todd EM, Roy SB, Hashimi AS, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation: A single-center experience in the present era. J Thorac Cardiovasc Surg 2017;154:1798-809.
4. Biscotti M, Gannon WD, Agerstrand C, et al. Awake Extracorporeal Membrane Oxygenation as Bridge to Lung Transplantation: A 9-Year Experience. Ann Thorac Surg 2017;104:412-9.
5. Ius F, Natanov R, Salman J, et al. Extracorporeal membrane oxygenation as bridge to lung transplantation may not impact overall mortality risk after transplantation: results from a 7-year single-centre experience. Eur J Cardiothorac Surg 2018. [Epub ahead of Print].
6. Hoetzenecker K, Donahoe L, Yeung JC, et al. Extracorporeal life support as a bridge to lung transplantation-experience of a high-volume transplant center. J Thorac Cardiovasc Surg 2018;155:1316-28.e1.
7. Hakim AH, Ahmad U, McCurry KR, et al. Contemporary Outcomes of Extracorporeal Membrane Oxygenation Used as Bridge to Lung Transplantation. Ann Thorac Surg 2018. [Epub ahead of print].
8. Bermudez CA, Shiose A, Esper SA, et al. Outcomes of Intraoperative Venoarterial Extracorporeal Membrane Oxygenation Versus Cardiopulmonary Bypass During Lung Transplantation. Ann Thorac Surg 2014;98:1936-42; discussion 1942-3.
9. Biscotti M, Yang J, Sonett J, et al. Comparison of extracorporeal membrane versus cardiopulmonary bypass for lung transplantation. J Thorac Cardiovasc Surg 2014;148:2410-5.
10. Machuca TN, Collaud S, Mercier O, et al. Outcomes of intraoperative extracorporeal membrane oxygenation versus cardiopulmonary bypass for lung transplantation. J Thorac Cardiovasc Surg 2015;149:1152-7.
11. Hoechter DJ, von Dossow V, Winter H, et al. The Munich Lung Transplant Group: Intraoperative Extracorporeal Circulation in Lung Transplantation. Thorac Cardiovasc Surg 2015;63:706-14.
12. Ius F, Sommer W, Tudorache I, et al. Five-year experience with intraoperative extracorporeal membrane oxygenation in lung transplantation: Indications and midterm results. J Heart Lung Transplant 2016;35:49-58.
13. Pettenuzzo T, Faggi G, Di Gregorio G, et al. Blood Products Transfusion and Mid-Term Outcomes of Lung Transplanted Patients Under Extracorporeal Membrane Oxygenation Support. Prog Transplant 2018. [Epub ahead of print].
14. Hoetzenecker K, Schwarz S, Muckenhuber M, et al. Intraoperative extracorporeal membrane oxygenation and the possibility of postoperative prolongation improve survival in bilateral lung transplantation. J Thorac Cardiovasc Surg 2018;155:2193-206.e3.
15. Hartwig MG, Walczak R, Lin SS, et al. Improved Survival but Marginal Allograft Function in Patients Treated With Extracorporeal Membrane Oxygenation After Lung Transplantation. Ann Thorac Surg 2012;93:366-71.
16. Tudorache I, Sommer W, Kühn C, et al. Lung Transplantation for Severe Pulmonary Hypertension–Awake Extracorporeal Membrane Oxygenation for Postoperative Left Ventricular Remodeling. Transplantation 2015;99:451-8.
17. Salman J, Ius F, Sommer W, et al. Mid-term results of bilateral lung transplant with postoperatively extended intraoperative extracorporeal membrane oxygenation for severe pulmonary hypertension. Eur J Cardiothorac Surg 2017;52:163-70.
18. Moser B, Jaksch P, Taghavi S, et al. Lung transplantation for idiopathic pulmonary arterial hypertension on intraoperative and postoperatively prolonged extracorporeal membrane oxygenation provides optimally

controlled reperfusion and excellent outcome. Eur J Cardiothorac Surg 2018;53:178-85.

19. Loor G, Simpson L, Parulekar A. Bridging to lung transplantation with extracorporeal circulatory support: when or when not? J Thorac Dis 2017;9:3352-61.

20. Rajagopal K, Hoeper MM. State of the Art: Bridging to lung transplantation using artificial organ support technologies. J Heart Lung Transplant 2016;35:1385-98.

21. Schaheen LW, D'Cunha J. Extracorporeal membrane oxygenation in lung transplantation: No longer a four-letter word. J Thorac Cardiovasc Surg 2018;155:2191-2.

22. Reeb J, Olland A, Renaud S, et al. Vascular access for extracorporeal life support: tips and tricks. J Thorac Dis 2016;8:S353-63.

23. Fernandez R, DeCamp M, Bharat A. A novel strategy for cardiopulmonary support during lung transplantation. J Thorac Dis 2018;10:E142-4.

24. Diaz-Guzman E, Sharma NS, Wille K, et al. Use of a novel pulmonary artery cannula to provide extracorporeal membrane oxygenation as a bridge to lung transplantation. J Heart Lung Transplant 2016;35:1051-3.

25. Bermudez CA, Zaldonis D, Fan MH, et al. Prolonged Use of the Hemolung Respiratory Assist System as a Bridge to Redo Lung Transplantation. Ann Thorac Surg 2015;100:2330-3.

26. Fischer S, Simon AR, Welte T, et al. Bridge to lung transplantation with the novel pumpless interventional lung assist device NovaLung. J Thorac Cardiovasc Surg 2006;131:719-23.

27. Strueber M, Hoeper MM, Fischer S, et al. Bridge to thoracic organ transplantation in patients with pulmonary artery hypertension using a pumpless lung assist device. Am J Transplant 2009;9:853-7.

28. Camboni D, Akay B, Pohlmann JR, et al. Veno-venous extracorporeal membrane oxygenation with interatrial shunting: a novel approach to lung transplantation for patients in right ventricular failure. J Thorac Cardiovasc Surg 2011;141:537-42.

29. Hartwig MG, Appel JZ III, Cantu E III, et al. Improved Results Treating Lung Allograft Failure With Venovenous Extracorporeal Membrane Oxygenation. Ann Thorac Surg 2005;80:1872-9; discussion 1879-80.

30. Chicotka S, Pedroso FE, Agerstrand CL, et al. Increasing Opportunity for Lung Transplant in Interstitial Lung Disease with Pulmonary Hypertension. Ann Thorac Surg 2018. [Epub ahead of print].

31. Glorion M, Mercier O, Mitilian D, et al. Central versus peripheral cannulation of extracorporeal membrane oxygenation support during double lung transplant for pulmonary hypertension. Eur J Cardiothorac Surg 2018. [Epub ahead of print].

32. Ius F, Sommer W, Tudorache I, et al. Veno-veno-arterial extracorporeal membrane oxygenation for respiratory failure with severe haemodynamic impairment: technique and early outcomes. Interact Cardiovasc Thorac Surg 2015;20:761-7.

33. Wong JK, Melvin AL, Joshi DJ, et al. Cannulation-Related Complications on Veno-Arterial Extracorporeal Membrane Oxygenation: Prevalence and Effect on Mortality. Artif Organs 2017;41:827-34.

34. Bisdas T, Beutel G, Warnecke G, et al. Vascular complications in patients undergoing femoral cannulation for extracorporeal membrane oxygenation support. Ann Thorac Surg 2011;92:626-31.

35. Bermudez CA, Rocha RV, Zaldonis D, et al. Extracorporeal membrane oxygenation as a bridge to lung transplant: midterm outcomes. Ann Thorac Surg 2011;92:1226-31.

36. Fuehner T, Kuehn C, Hadem J, et al. Extracorporeal membrane oxygenation in awake patients as bridge to lung transplantation. Am J Respir Crit Care Med 2012;185:763-8.

37. Lang G, Taghavi S, Aigner C, et al. Primary lung transplantation after bridge with extracorporeal membrane oxygenation: a plea for a shift in our paradigms for indications. Transplantation 2012;93:729-36.

38. Javidfar J, Brodie D, Iribarne A, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation and recovery. J Thorac Cardiovasc Surg 2012;144:716-21.

39. Toyoda Y, Bhama JK, Shigemura N, et al. Efficacy of extracorporeal membrane oxygenation as a bridge to lung transplantation. J Thorac Cardiovasc Surg 2013;145:1065-70.

40. Lafarge M, Mordant P, Thabut G, et al. Experience of extracorporeal membrane oxygenation as a bridge to lung transplantation in France. J Heart Lung Transplant 2013;32:905-13.

41. Dellgren G, Riise GC, Swärd K, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation: a long-term study. Eur J Cardiothorac Surg 2015;47:95-100.

42. Yeo HJ, Lee S, Yoon SH, et al. Extracorporeal Life Support as a Bridge to Lung Transplantation in Patients with Acute Respiratory Failure. Transplant Proc 2017;49:1430-5.

43. Bittner HB, Lehmann S, Rastan A, et al. Outcome of

extracorporeal membrane oxygenation as bridge to lung transplantation and graft recovery. Ann Thorac Surg 2012;94:942-9.

44. Schmidt F, Sasse M, Boehne M, et al. Concept of "awake venovenous extracorporeal membrane oxygenation" in pediatric patients awaiting lung transplantation. Pediatr Transplant 2013;17:224-30.

45. Lang G, Kim D, Aigner C, et al. Awake extracorporeal membrane oxygenation bridging for pulmonary retransplantation provides comparable results to elective retransplantation. J Heart Lung Transplant 2014;33:1264-72.

46. Hayanga AJ, Aboagye J, Esper S, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation in the United States: an evolving strategy in the management of rapidly advancing pulmonary disease. J Thorac Cardiovasc Surg 2015;149:291-6

47. Hayes D Jr, Tobias JD, Tumin D. Center Volume and Extracorporeal Membrane Oxygenation Support at Lung Transplantation in the Lung Allocation Score Era. Am J Respir Crit Care Med 2016;194:317-26.

48. Schechter MA, Ganapathi AM, Englum BR, et al. Spontaneously Breathing Extracorporeal Membrane Oxygenation Support Provides the Optimal Bridge to Lung Transplantation. Transplantation 2016;100:2699-704.

49. Tsiouris A, Budev MM, Yun JJ. Extracorporeal Membrane Oxygenation as a Bridge to Lung Transplantation in the United States: A Multicenter Survey. ASAIO J 2017. [Epub ahead of print].

50. Turner DA, Cheifetz IM, Rehder KJ, et al. Active rehabilitation and physical therapy during extracorporeal membrane oxygenation while awaiting lung transplantation: a practical approach. Crit Care Med 2011;39:2593-8.

51. Rahimi RA, Skrzat J, Reddy DRS, et al. Physical Rehabilitation of Patients in the Intensive Care Unit Requiring Extracorporeal Membrane Oxygenation: A Small Case Series. Phys Ther 2013;93:248-55.

52. Hayes K, Hodgson CL, Pellegrino VA, et al. Physical Function in Subjects Requiring Extracorporeal Membrane Oxygenation Before or After Lung Transplantation. Respir Care 2018;63:194-202.

53. Kolaitis NA, Soong A, Shrestha P, et al. Improvement in patient-reported outcomes after lung transplantation is not impacted by the use of extracorporeal membrane oxygenation as a bridge to transplantation. J Thorac Cardiovasc Surg 2018;156:440-8.e2.

54. Hayanga AJ, Du AL, Joubert K, et al. Mechanical

Ventilation and Extracorporeal Membrane Oxygenation as a Bridging Strategy to Lung Transplantation: Significant Gains in Survival. Am J Transplant 2018;18:125-35.

55. Ius F, Kuehn C, Tudorache I, et al. Lung transplantation on cardiopulmonary support: Venoarterial extracorporeal membrane oxygenation outperformed cardiopulmonary bypass. J Thorac Cardiovasc Surg 2012;144:1510-6.

56. Aigner C, Wisser W, Taghavi S, et al. Institutional experience with extracorporeal membrane oxygenation in lung transplantation. Eur J Cardiothorac Surg 2007;31:468-73; discussion 473-4.

57. Yu WS, Paik HC, Haam SJ, et al. Transition to routine use of venoarterial extracorporeal oxygenation during lung transplantation could improve early outcomes. J Thorac Dis 2016;8:1712-20.

58. Cosgun T, Tomaszek S, Opitz I, et al. Single-center experience with intraoperative extracorporeal membrane oxygenation use in lung transplantation. Int J Artif Organs 2017. [Epub ahead of print].

59. Magouliotis DE, Tasiopoulou VS, Svokos AA, et al. Extracorporeal membrane oxygenation versus cardiopulmonary bypass during lung transplantation: a meta-analysis. Gen Thorac Cardiovasc Surg 2018;66:38-47.

60. Hoechter DJ, Shen YM, Kammerer T, et al. Extracorporeal Circulation During Lung Transplantation Procedures: A meta-Analysis. ASAIO J 2017;63:551-61.

61. Ribeiro Neto ML, Budev M, Culver DA, et al. Venous Thromboembolism After Adult Lung Transplantation: A Frequent Event Associated With Lower Survival. Transplantation 2018;102:681-7.

62. Narm KS, Lee S, Suh JW, et al. Risk Factor Analysis for Intraoperative Extracorporeal Membrane Oxygenation Weaning Failure After Lung Transplantation. Ann Thorac Surg 2018;105:242-8.

63. Vlasselaers D, Verleden GM, Meyns B, et al. Femoral Venoarterial Extracorporeal Membrane Oxygenation for Severe Reimplantation Response After Lung Transplantation. Chest 2000;118:559-61.

64. Meyers BF, Sundt TM III, Henry S, et al. Selective use of extracorporeal membrane oxygenation is warranted after lung transplantation. J Thorac Cardiovasc Surg 2000;120:20-6.

65. Dahlberg PS, Prekker ME, Herrington CS, et al. Medium-Term Results of Extracorporeal Membrane Oxygenation for Severe Acute Lung Injury After Lung Transplantation. J Heart Lung Transplant 2004;23:979-84.

66. Oto T, Rosenfeldt F, Rowland M, et al. Extracorporeal Membrane Oxygenation After Lung Transplantation:

Evolving Technique Improves Outcomes. Ann Thorac Surg 2004;78:1230-5.

67. Mason DP, Boffa DJ, Murthy SC, et al. Extended use of extracorporeal membrane oxygenation after lung transplantation. J Thorac Cardiovasc Surg 2006;132:954-60.

68. Fischer S, Bohn D, Rycus P, et al. Extracorporeal membrane Oxygenation for Primary Graft Dysfunction After Lung Transplantation: Analysis of the Extracorporeal Life Support Organization (ELSO) Registry. J Heart Lung Transplant 2007;26:472-7.

69. Bermudez CA, Adusumilli PS, McCurry KR, et al. Extracorporeal Membrane Oxygenation for Primary Graft Dysfunction After Lung Transplantation: Long-Term Survival. Ann Thorac Surg 2009;87:854-60.

70. Marasco SF, Vale M, Preovolos A, et al. Institution of extracorporeal membrane oxygenation late after lung transplantation–a futile exercise? Clin Transplant 2012;26:E71-7.

71. Mulvihill MS, Yerokun BA, Davis RP, et al. Extracorporeal membrane oxygenation following lung transplantation: indications and survival. J Heart Lung Transplant 2017;37:259-67.

72. Snell GI, Yusen RD, Weill D, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction, part I: Definition and grading–A 2016 Consensus Group statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2017;36:1097-103.

73. Shah RJ, Diamond JM. Primary Graft Dysfunction (PGD) Following Lung Transplantation. Semin Respir Crit Care Med 2018;39:148-54.

74. Porteous MK, Ky B, Kirkpatrick JN, et al. Diastolic Dysfunction Increases the Risk of Primary Graft Dysfunction after Lung Transplant. Am J Respir Crit Care Med 2016;193:1392-400.

Machine perfusion of thoracic organs

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Abstract: This article summarizes recent knowledge and clinical advances in machine perfusion (MP) of thoracic organs. MP of thoracic organs has gained much attention during the last decade. Clinical studies are investigating the role of MP to preserve, resuscitate, and assess heart and lungs prior to transplantation. Currently, MP of the cardiac allograft is essential in all type DCD heart transplantation while MP of the pulmonary allograft is mandatory in uncontrolled DCD lung transplantation. MP of thoracic organs also offers an exciting platform to further investigate downregulation of the innate and adaptive immunity prior to reperfusion of the allograft in recipients. MP provides a promising technology that allows pre-transplant preservation, resuscitation, assessment, repair, and conditioning of cardiac and pulmonary allografts outside the body in a near physiologic state prior to planned transplantation. Results of ongoing clinical trials are awaited to estimate the true clinical value of this new technology in advancing the field of heart and lung transplantation by increasing the total number and the quality of available organs and by further improving recipient early and long-term outcome.

Keywords: Heart transplantation; lung transplantation; machine perfusion (MP); *ex vivo* lung perfusion (EVLP); donor after circulatory death

Introduction

"*Good morning doctor, I hope you had a good night of sleep. The organ has been fully reconditioned overnight. It has now been assessed and accepted for transplantation. We have called upon the recipient for transfer to the operating room*" said the scrub nurse to the surgeon over the phone. This is a scenario every transplant surgeon would dream of to become reality during his professional career.

Heart as well as lung transplantation have become a standard life-saving therapy in selected patients suffering from end-stage heart (1) or lung (2) failure. In addition, quality of life is remarkably improved in the majority of these recipients. Selection criteria for heart (3) and lung (4)

transplant candidates have recently been reviewed by working groups within the International Society for Heart and Lung Transplantation (ISHLT). However, the application of this ultimate treatment modality is currently limited by the number of "acceptable" organ donors and "transplantable" grafts.

During the last decade, machine perfusion (MP) of solid organs has become clinical reality and offers the possibility to assess, preserve and recondition organs prior to transplantation. Previous review papers have reported on the different techniques, protocols and devices currently available for perfusion of heart (5-9) and lungs (10-21). Increasing comfort with this new technology and important clinical experience with MP was reported over the last

24 months. The purpose of this review is to summarize the recent clinical experience in the perspective of future clinical applications.

MP of thoracic organs for normothermic preservation

To slow down metabolism and reduce warm ischemic injury, organs nowadays are cooled by flushing them with organ specific preservation solutions followed by *static* cold storage (SCS). Cooling from 37 to 4 ℃ decreases the metabolic rate by 12- to 13-fold. This method was designed at a time when donors were "ideal" and preservation periods were short. Up to date it remains the golden standard for preservation of heart (22,23) and lungs (24,25).

In an era of donor shortage, increased use of suboptimal grafts, and organ exchange across sometimes distant geographical areas, SCS has reached its limits. Although the technique of cold storage is simple, safe, and cheap, metabolism does not cease completely. Anoxia may still occur and sodium-potassium ATPases are still inhibited with disruption of transcellular ion gradients. Primary graft dysfunction (PGD) or even primary non-function of heart (26) and lungs (27) jeopardizing the life of the recipient immediately after transplantation remains a substantial risk. Time has come to think outside the ice-box (28).

Indeed, MP has the potential to better preserve graft quality by sustaining continued metabolism dependent on the perfusion temperature, by providing oxygen, energy and nutrients, and by removing toxic waste products (in case the perfusate is regularly renewed or filtered). Over the last decade, old techniques dating from the early days of transplantation have re-emerged whereby organs are continuously and *dynamically* perfused instead of being *statically* cold stored during their preservation. This technology was substantially refined and new devices for MP of heart and lungs are being developed and introduced in the clinical arena. Portable systems will largely facilitate continuous MP preservation during transport between donor and recipient hospitals.

Heart

In the past several decades there has been scientific and clinical interest towards *ex situ* heart perfusion with oxygenated and nutrient enriched blood to reduce ischemic injury to the donor heart and potentially enable assessment of metabolic and mechanical function. Hypothermic MP

of heart was reported in animal experiments with long preservation times (29-33). Recent preclinical studies with hypothermic MP confirm that it provides superior donor heart preservation compared to cold static storage in terms of left ventricular function, cardiac myocyte integrity, and energy stores (34-39). Hypothermic MP devices have been developed for human heart preservation. After a set of experiments in a porcine heart transplant model reported by Steen *et al.* (33), a clinical trial in humans is now ongoing at the Skåne University Hospital, Lund, Sweden. The first human heart transplant using Stig Steen's new heart solution and machine has been successful [Steen S (Lund, Sweden) personal communication].

Several reports have investigated normothermic MP of the donor heart to maintain a steady state of metabolism (7,40-42). An elevated lactate level at the end of MP appears to be a powerful predictor of graft failure (43). The feasibility of normothermic *ex situ* heart perfusion for 12 hours has previously been demonstrated with recovery of cardiac function and preservation of endothelial cell function (44,45). These studies have paved the way for development of clinical devices for *ex situ* heart perfusion.

The Organ Care System (OCS) is the first and only clinical platform up to date that can maintain the donor heart in a warm, beating, near-physiological state prior to transplantation (OCS™ Heart, Transmedics®, Andover, MA, USA). Institutional studies reported the successful use of the OCS in human heart transplantation (46-48). Clinical trials were started in 2007 in USA (PROCEED) (49) and in Europe (PROTECT) (50) with results presented in abstract form only. In 2015, Ardehali *et al.* reported the results of the first clinical trial (PROCEED II) in heart transplantation to assess the efficacy and safety of this new technology (51). In a prospective, open-label, multicenter, randomized non-inferiority trial (ClinicalTrials.gov, number NCT00855712) at ten heart-transplant centers in the USA and Europe, heart-transplant candidates (aged >18 years) were randomized to receive donor hearts preserved with either the OCS (n=67) or SCS (n=63) (52). Thirty-day patient and graft survival rates were 94% and 97%, respectively (P=0.45). Eight (13%) patients in the OCS group and nine (14%) patients in the SCS group had cardiac-related serious adverse events. The authors concluded that OCS yield similar short-term clinical outcomes (51). In an editorial commentary in Lancet, the clinical value of this new technology for standard heart preservation was however questioned. Some hearts that looked initially acceptable for transplantation were ultimately not implanted. OCS

also requires additional surgical and technical support, proprietary equipment, and appropriate transport that are inevitably more costly than those needed for cold static storage (53). However, proponents believe that the *ex situ* perfusion of the heart is able to enhance viability of donor organs by reducing time-dependent ischemic injury (54). In a single-center, non-randomized study, better outcomes with this new technology compared to SCS were reported with regard to recipient survival and incidence of PGD as well as acute rejection (46). Further studies are needed to evaluate the impact of this new preservation technology on the number of heart transplants and its outcome.

Lung

Compared to heart transplantation, the impact of the length of the cold ischemic time on the outcome after lung transplantation is less clear. Over the years several studies with inherent flaws have reported conflicting results on this topic (2,55,56). With modern extracellular-type preservation solutions, lungs preserved on ice can be safely transplanted within a time window of 8–10 hours. Yeung *et al.* from the Toronto group recently reported that the extension of graft preservation time beyond 12 hours with EVLP did not negatively affect early lung transplantation outcomes (57).

Ex vivo lung perfusion (EVLP) was reported in historical papers as a method to assess the quality of the graft (58) and as preservation technique during distant thoracic organ procurement (40). The first successful transplant after EVLP was published by Steen and colleagues in 2001 (59). Much experimental work on the technique for prolonged EVLP was carried out at the Universities of Lund (60) and Toronto (61). The attention of most research groups was mainly focused on the value of EVLP as a tool to assess the quality of non-standard lungs prior to acceptance for transplantation (13-21). Because of the comfort and the good outcome with SCS, little clinical interest was shown in this technology as a potential tool for normothermic lung preservation (62). A prospective, international, multicenter, randomized controlled, non-inferiority clinical study (Inspire trial) was recently completed comparing normothermic portable *ex vivo* machine preservation with the OCS Lung™ (Transmedics®, Andover, MA, USA) to SCS of standard donor lungs (Clinical Trials.gov number NCT 01630434) (63). A total of 320 patients were randomized to both treatment arms. This is the largest clinical and randomized trial in lung preservation performed to date. The primary effectiveness end-point was a composite of patient and graft survival at day 30 and absence of PGD grade 3 within the first 72 hours. The final results were presented at the 2016 annual ISHLT meeting (64). The study showed that the OCS group met the non-inferiority test as compared to the SCS group in the per protocol population. Of notice, the incidence of PGD grade 3 within 72 hours after transplantation in that population was significantly lower (P=0.015) in the OCS group. The investigators stated that this finding might have an impact on the development of chronic rejection and long-term survival, but this will need further study follow-up. The full paper reporting study results is still awaited.

While normothermic *dynamic* preservation of donor lungs on the portable OCS Lung™ device in the Inspire trial already commenced in the donor hospital and continued during transport to the recipient hospital, other groups have looked at the value of normothermic *static* preservation after a first cold ischemic period prior to transplantation. In a pig lung transplant model, the Toronto group previously investigated the impact of prolonged (12 hours) normothermic EVLP following a first period of 12 hours cold ischemia. Recipient animals did better in terms of superior oxygenation and less edema when compared to recipients of lungs that were stored cold for 24 hours (62). In a recent study using the same transplant model, this group investigated the impact of a second cold ischemic period (2 and 10 hours) following a first 10-hour period of cold storage and then 6 hours of normothermic EVLP. After 4 hours of reperfusion in the recipient animal, oxygenation function, acute lung injury score, inflammatory markers, and cell death pathway markers were similar between the 2- and 10-hour groups. Of notice, both EVLP groups demonstrated better oxygenation compared to the control group with 24 hours cold static preservation without EVLP (65). A prospective, single-center clinical trial was conducted by the Vienna lung transplant team randomizing 80 patients transplanted with cold stored lungs immediately upon arrival versus similar lungs that were first evaluated for 4 hours with normothermic static EVLP using the Toronto technique (66). Short-term clinical outcomes in recipients did not differ between both groups. Patients remained intubated (1.6 *vs.* 1.6 days, P=0.67), in the intensive care unit (6 *vs.* 6 days, P=0.76), and in the hospital (23 *vs.* 19 days, P=0.42) for a comparable period of time. The 30-day survival was 97.1% *vs.* 100% (P=0.46). Of note, the incidence of PGD more than grade 1 was lower in the EVLP group at all-time points compared to the control group, but this difference failed to reach statistical

significance (24 hours, 5.7% *vs.* 19.5%, P=0.10). Likewise, the need for post-operative prolonged extracorporeal membrane oxygenation was lower in the EVLP group (5.7% *vs.* 12.2%, P=0.44).

Further studies are needed to evaluate the impact of this new preservation technology and its best timing in the total preservation process on the outcome after lung transplantation.

MP of thoracic organs for transplantability assessment

In addition to the potential of safely replacing and prolonging the preservation period, MP creates a "window" between procurement and transplantation during which real-time functional performance, metabolic need, and viability of the graft can be evaluated under optimal conditions. Data collected during this preservation period may provide information that can help clinicians to predict the risk of PGD and that can assist them in deciding to accept or discard a given organ for transplantation. This new platform, therefore, may provide a tool to select "transplantable" grafts of the best quality in an effort to increase the thoracic donor organ pool. In a retrospective database analysis of declined lung donors, our group identified a large potential (>20%) for EVLP to further increase the donor pool in a transplant center where the majority of donor lungs are already fulfilling extended criteria (67). Similarly, MP of heart is expected to significantly increase the total number of hearts accepted for transplantation (5,54).

Heart

The Harefield group reported on the successful use of OCS to assess heart quality in transplantation from donors with an adverse profile (e.g., left ventricular ejection fraction <50%, left ventricular hypertrophy, donor cardiac arrest, alcohol/drug abuse, coronary artery disease) (68). The International Expand Heart Pivotal Trial (Clinical Trials. gov number NCT 02323321) is currently investigating this potential with donor hearts that do not meet current standard acceptance criteria (69). Results of the trial are awaited.

After intensive research in large animal models (70-75), recent success with clinical heart transplantation from donors dying after circulatory arrest (DCD) has boosted the interest in normothermic *ex situ* heart perfusion as a tool to assess cardiac recovery after hypoxic arrest and subsequent functional performance prior to transplantation (7,76). The groups in Sydney (77-79), Cambridge (80,81), and Harefield (68) have now reported case series of DCD heart transplantation with excellent early survival. A new method to assess performance of the heart recovered from a DCD was recently reported by the group at Papworth Hospital, Cambridge, UK. Extended thoracoabdominal normothermic regional perfusion (NRP) in the deceased donor with the aid of venoarterial extracorporeal membrane oxygenation allows metabolic and functional recovery and subsequent assessment of the arrested heart *in situ*. In their opinion, donor hearts that fail post-NRP assessment can be discarded avoiding the use of expensive material for *ex situ* functional evaluation (82). The authors speculate that thoraco-abdominal NRP may become the new gold standard for DCD organ retrieval in the future.

Lung

Equally, MP allows quality assessment of the pulmonary graft prior to transplantation. The first successful transplant after EVLP in 2000 was with a lung recovered from an uncontrolled DCD (59). More interest in EVLP was noticed for pulmonary grafts that initially did not meet standard lung criteria. Successful transplantation of questionable lungs after EVLP has now been reported by several groups in Europe and North America with good clinical outcome (83-99). The overall lung yield after EVLP across all reported series is around 80% (17).

The role of EVLP for secondary assessment of questionable donor lungs is being investigated in several clinical trials (100). The first clinical trial was conducted in Canada by the Toronto Lung Transplant Group. In the *HELP* trial (Human *Ex Vivo* Lung Perfusion), high-risk lungs that otherwise would not be used, were assessed with EVLP. Eighty six percent of the lungs that originally did not meet acceptance criteria from both DBDs and DCDs, were ultimately transplanted after EVLP and resulted in equivalent recipient outcome compared to those of contemporary standard control donor lungs. Rates of PGD grade 3 at 72 hours after transplantation were reported to be low (2% in EVLP lungs versus 8.5% in control lungs) (84,88). More than 100 clinical lung transplants have now been performed in Toronto with a 5-year survival of 70% in the EVLP cohort compared to 63% in controls (100). Functional outcome and quality of life are equivalent to

conventional lung transplants (101). The *DEVELOP*-UK trial including all five lung transplant centers in the UK (Controlled Trials.com number ISRCTN44922411) was designed to compare one-year recipient survival between standard-criteria (SCD) versus extended-criteria (ECD) donor lungs after EVLP reconditioning according to the Lund protocol using the Vivoline® LS1 device (XVivo Perfusion AB, Göteborg, Sweden) (102). The trial started in April 2012, but was prematurely stopped after some fatalities. Results have been reported recently (103). Overall, one-third of donor lungs subjected to EVLP were deemed suitable for transplant. Estimated survival over 12 months was lower than in the standard group, but the data were also consistent with no difference in survival between groups. Patients receiving these additional transplants experienced a higher rate of early graft injury and need for unplanned ECMO support, at increased cost. Three multicenter trials are still ongoing. The *NOVEL* trial (Clinical Trials.gov number NCT 01365429) is a prospective, non-randomized, controlled, clinical study in 104 recipients in eight U.S. centers comparing 30-day post-transplant mortality as primary end-point between SCD versus ECD lungs after EVLP reconditioning according to the Toronto protocol using the XPS™ device (XVivo Perfusion AB, Göteborg, Sweden) (104). The trial was started in May 2011 and is still recruiting patients. Preliminary results were updated at the 2014 annual ISHLT Meeting (105). The *Expand* Lung Trial (Clinical Trials.gov number NCT 01963780) is a prospective, international, multicenter, non-randomized, single-arm clinical study that examines the safety and effectiveness of the OCS™ Lung perfusion device for recruiting, preserving, and assessing ECD lungs for transplantation (106). Preliminary results on the first cases were presented at the 2014 (107) and 2016 (108) annual ISHLT meetings. The trial is now completed and final results are awaited. Finally, the *Perfusix* trial (Clinical Trials.gov number NCT 02234128) in the US is looking at extending preservation and assessment time of donor lungs using the Toronto EVLP System™. Retrieved lungs will be shipped to a dedicated EVLP facility (109).

The routine or selective use of EVLP for controlled DCD lung evaluation is still controversial as good outcome has been reported without EVLP (110,111). The Toronto group compared the outcome after DCD lung transplantation with and without EVLP. Survival was comparable although EVLP cases had a shorter length of ventilation and hospitals stay (112). The authors concluded that EVLP helped to safely increase their DCD lung

utilization. In our own experience, controlled DCD lung transplantation with a short (<30 min) total warm ischemic time results in excellent short- and long-term outcome without using EVLP (113). EVLP can be performed in case of any doubt of graft quality (112,114). For uncontrolled DCD, however, EVLP is indispensable to evaluate graft quality since there is no clinical information available before the arrival of the retrieval team. Also, the incidence of PGD grade 3 is expected to be higher after transplantation as previously reported by the Madrid group (115,116). Other groups have followed a similar policy of pre-transplant lung assessment from such donors (117,118).

MP of thoracic organs for repair and reconditioning

As discussed above, MP creates a "window" between procurement and transplantation during which functional performance and viability of the graft can be evaluated. If prolonged dynamic preservation (>12–24 hours) of thoracic organs proves to be feasible and safe, MP may offer a tool for *ex vivo* repair and quality improvement prior to transplantation, thereby not forgetting the importance of *in vivo* optimization prior to organ procurement (119). Many organs, excluding those with fixed structural damage related to previous injuries or life-style habits such as smoking or alcohol abuse, are currently declined because of acute—albeit recoverable—damage. Thoracic organs may get injured by several hits during the whole transplantation process in the transition phase from donor to recipient. Altogether, organ damage may result from direct trauma, inflammation, infection, brain death and the agonal phase and warm ischemia in a DCD setting.

Once the organs are recovered from the deceased body, *ex situ* treatment during MP theoretically becomes possible (120,121). Intravascular perfusion providing oxygen and other metabolic substrates under physiological conditions appears to be the way forward to improve the viability of suboptimal grafts and may already be sufficient to recover intrinsic repair mechanisms. Additional specific treatments targeting different pathways to interfere with the organ have been suggested (100). The easiest strategy would be to deliver drugs directly to the organs by including them into the perfusion solution or by injecting active agents into the afferent tubing running to the vasculature of the graft. Theoretically, pharmacological interventions could be targeted according to the type of injury or even given in combination as a "cocktail" at intervals during MP:

anti-bacterial, anti-viral, and anti-fungal agents to treat infection (122,123), anti-inflammatory molecules to block pro-inflammatory responses (124-126), cytoprotective and anti-ischemic metabolic agents (127-129), agents initiating or enhancing ischemic postconditioning, vasodilating agents to improve perfusion of the microvasculature, fibrinolytic agents to dissolve microthrombi (130), dehydration of tissue with perfusate with high oncotic pressure, etc. An advantage of this isolated MP setting is that these drugs could be given at higher doses than *in vivo* since there is no risk to harm other organs. A restriction, however, may be that certain drugs cannot be metabolized in the circuit and therefore active components would have to be given. On the other hand, toxic metabolites may accumulate over time. Therefore, repeated renewal of the perfusate, hemofiltration, or insertion of filters and membranes in the circuit may become necessary for removal of harmful and toxic waste products (blood clots, neutrophils, inflammatory cytokines). Finally, MP also offers the possibility to interfere at the genetic level by using viral vectors (131) or silencing RNA technology. The aforementioned organ repair strategies during MP are currently experimental and only very few clinical papers have been published so far. In the future, organ reconditioning hubs may appear to be an efficient method of delivering this service to all transplant centers (132).

Heart

Beside the series reporting on MP to evaluate the quality of DBD hearts (68) or to resuscitate DCD hearts (77,81), the authors are not aware of any clinical study or case report whereby an initially unacceptable cardiac allograft was first rehabilitated *ex situ* during MP prior to transplantation.

Research is ongoing to investigate the best conditions in terms of perfusate and active agents during MP of the heart (133,134).

Lung

Compared to other solid organs, the lung can be considered as a privileged organ as it not only carries a vascular, but also a bronchial tree providing direct access to the entire parenchyma. In that way, drugs or gases can be delivered to the pulmonary graft by instillation or inhalation (100). *Ex situ* administration of surfactant via lavage was demonstrated to improve graft function of acid-injured lungs in a porcine EVLP model (135). In a recent study by

our group, no beneficial effect of ventilation with the inert gas argon during EVLP could be demonstrated in a porcine model (136). Ventilation of the pulmonary allograft with an inhaled bronchodilator during EVLP improved lung graft function after transplantation in a canine model (137).

Debate continues about the best conditions for EVLP with regards to cellular versus acellular composition of the perfusate (138-141), the importance of left atrial pressure (138,142), positive versus negative pressure ventilation (143), the use of leucocyte (144) or cytokine filters (145,146) in the circuit, the oxygenation level of the perfusate (147), and the role of hemofiltration (148). Research is ongoing to identify clinical biomarkers in the perfusate such as cytokines (149), endothelial markers (150), adhesion molecules (151), metabolomics (152) and to investigate imaging techniques (153,154) before and after EVLP that may be predictive of graft function after transplantation.

Few clinical case reports on successful transplantation of rehabilitated pulmonary grafts have been published so far. Sanchez *et al.* reported successful outcome after transplantation of a salvaged lung that was first reconditioned during MP for neurogenic pulmonary edema (155). Both the Zurich group (156) and the Toronto (157) reported on a case of pulmonary thrombolysis during MP followed by successful lung transplantation.

MP of thoracic organs to downregulate allograft immunity

Beside *ex vivo* repair and quality improvement, MP may offer a tool for "immunoregulation" of thoracic organs in order to protect them from responses related to the innate (ischemia-reperfusion injury) and adaptive (acute and chronic rejection) immunity developing in the recipient.

Heart

To the authors' knowledge, no studies have been reported so far that investigated the role of MP to improve immune tolerance of the heart in the recipient after transplantation.

Lung

Two interesting studies exploring the impact of donor passenger antigen-presenting leucocytes on immunogenicity were reported recently. In a first study by Stone et al, passenger leukocyte migration from donor lungs into the

recipient and the effects of donor leukocyte depletion during EVLP were investigated in a gender-mismatched porcine lung transplant model. Donor leukocyte transfer into the recipient and migration to recipient lymph nodes were markedly reduced in the group receiving EVLP lungs compared to a control group transplanted with standard lungs. In addition, recipient T cell infiltration of the donor lung was significantly diminished in the study group (158). In another study by Noda *et al.*, the role of circulating leukocytes in lungs and their relationship with circulating pro-inflammatory cytokines on ischemia-reperfusion injury was investigated in a rat lung transplant model (159). Lung function was significantly better in lung grafts on EVLP with a leucocyte filter in the circuit compared to a control group without. Interleukin-6 levels in pulmonary grafts and in perfusate were also significantly lower after EVLP in the study group. After transplantation, graft function was better and inflammatory response was less. From both studies, it appears (I) that passenger donor leucocytes play an important role in the innate and adaptive alloreactivity; and (II) that EVLP including a leucocyte filter in the circuit may be a therapeutic approach to reduce the immune response. Interestingly, in a retrospective analysis of the clinical experience with EVLP in Toronto, the authors reported that EVLP assessed lungs from brain-dead donors (DBD) developed less chronic rejection (101).

Another interesting approach to condition the graft may be the use of mesenchymal stem or stromal cells (MSCs) during MP (160). These cells are multipotent self-renewing cells isolated from whole bone marrow. A paradigm shift has occurred in our concept of how cell therapies utilizing MSCs mediate their beneficial effects. It is now appreciated that, although MSCs can be described as having differentiation potential, their effector function is based less on *in situ* differentiation, trans-differentiation, or fusion and more on paracrine effects and cross-talk with other cells within diseased tissues. Mechanistic hypotheses of MSCs as cell-based therapy are postulated on their immunoregulatory properties (interaction with the innate immunity and suppression of T-cell responses) and their ability to secrete soluble factors or microspheres (161). These properties of MSCs make them particularly interesting for use as a cellular therapy in solid-organ transplantation (162,163). MP offers a unique platform to selectively administer these MSCs directly into the donor organ overcoming issues of homing, trafficking and safety. Especially allogeneic MSCs are attractive due to their wide availability at the time of organ harvest. Autologous stem

cells might be of less interest to modulate acute donor organ injury during MP since the isolation steps take longer time intervals and can never be planned in advance when a potential donor becomes available.

In lung, much research was done by the group at the University of California at San Francisco (164,165). Several basic anti-inflammatory and anti-bacterial properties have been attributed to MSCs and their extracellular vesicles that may be beneficial to restore epithelial and endothelial permeability in patients with acute lung injury from trauma or sepsis comparable to donor lung injury after reperfusion (166).

The spectrum of possible MSCs-based therapies for donor lung injury includes both targeted intrapulmonary and intravascular administration during EVLP. This was investigated in two recent studies using a porcine EVLP model. In a first study on the optimal route and dose for administering MSCs reported by the Toronto group, intravascular administration of 50×10^6 MSCs was associated with significant and sustained retention of MSCs in lung parenchyma, whereas intra-bronchial administration was not. Intravascular administration of 150×10^6 MSCs was the optimal tolerated dose and was associated with increased concentrations of human vascular endothelial growth factor in lung biopsies and decreased concentrations of pig interleukin-8 in the perfusate during 12 hours of EVLP (167). In another study by the Leuven group, the immunoregulatory capacities of multipotent adult progenitor cells (MAPC) on PGD were investigated in a lung injury model when administered via the airways. Although physiologic parameters during 6 hours EVLP were not different between both study groups, neutrophilia in bronchoalveolar lavage (BAL) fluid was significantly reduced in the MAPC group compared to controls, accompanied with a significant decrease in TNF-α, IL-1β and IFN-γ in the BAL (168).

Many issues related to MSCs therapy in transplantation (cell type, timing and route of administration, trafficking and homing) remain unresolved and warrant further research. MP provides a unique tool to deliver these therapies directly to thoracic organs while they remain physiologically perfused and metabolically active in an isolated circuit.

If the above would prove to be possible, this may revolutionize the practice of solid organ transplantation by increasing the number of transplantable grafts and by improving their function and facilitating their acceptance post-transplant thereby reducing the need for immunosuppression and its attending complications (toxicity, infection and malignancies) (169-171).

Conclusions

MP of thoracic organs has gained much attention during the last decade. So far, clinical research has been focused on MP for prolonged preservation of standard hearts and lungs as a tool to increase the cross-clamp time and to reduce early graft dysfunction in the recipient. In addition, MP of heart has become an essential tool to resuscitate and to evaluate the quality of the cardiac allograft from a DCD. The largest clinical experience with MP of lung was reported as a tool to evaluate functional performance of questionable lungs prior to transplantation. MP prior to transplanting lungs from a controlled DCD with a short total warm ischemic time is probably not essential, but MP is indispensable to evaluate lung graft quality from an uncontrolled DCD. Clinical experience with MP to repair and treat previously unacceptable lungs is limited to case reports. The use of MP as an immunoregulating tool for inducing better tolerance of the thoracic organ in the recipient after transplantation is exciting and hopeful.

Further research is needed to establish the best method and preservation solutions for long-term MP. The jury is still out if MP will have an impact on long-term survival in addition to the current promising short-term results. The outcome of ongoing clinical studies is awaited to delimit the proper indications before MP will become a routine method in our daily transplant practice.

Footnote

Conflicts of Interest: D Van Raemdonck was a principal investigator for the Inspire Trial and for the Expand Trial; both trials were sponsored by Transmedics® (Andover, MA, USA). F Rega receives research grants from Medtronic and is a consultant to Atricure Europe (Amsterdam, the Netherlands) and LivaNova Belgium (Zaventem, Belgium). The other authors have no conflicts of interest to declare.

References

1. Lund LH, Khush KK, Cherikh WS, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. J Heart Lung Transplant 2017;36:1037-46.
2. Chambers DC, Yusen RD, Cherikh WS, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-fourth adult lung and heart-lung transplantation report-2017; focus theme: allograft ischemic time. J Heart Lung Transplant 2017;36:1047-59.
3. Mehra MR, Canter CE, Hannan MM, et al. The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: A 10-year update. J Heart Lung Transplant 2016;35:1-23.
4. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.
5. Messer S, Ardehali A, Tsui S. Normothermic donor heart perfusion: current clinical experience and the future. Transpl Int 2015;28:634-42.
6. Dhital K, Simon A, Macdonald P. Ex-vivo donor heart perfusion: testing the limits of cardiac resilience. Heart Lung Circ 2015;24:1191-2.
7. Macdonald PS, Chew HC, Connellan M, et al. Extracorporeal heart perfusion before heart transplantation: the heart in a box. Curr Opin Organ Transplant 2016;21:336-42.
8. Fukushima N. Current status and future aspects of ex vivo allograft perfusion for heart transplantation. J Heart Lung Transplant 2017;36:247-9.
9. Hasan R, Ela AA, Goldstein D. Innovations in cardiac transplantation. Curr Opin Cardiol 2017. [Epub ahead of print].
10. Yeung JC, Cypel M, Waddell TK, et al. Update on donor assessment, resuscitation, and acceptance criteria, including novel techniques--non-heart-beating donor lung retrieval and ex vivo donor lung perfusion. Thorac Surg Clin 2009;19:261-74.
11. Cypel M, Keshavjee S. Extracorporeal lung perfusion. Curr Opin Organ Transplant 2011;16:469-75.
12. Cypel M, Keshavjee S. The clinical potential of ex vivo lung perfusion. Expert Rev Respir Med 2012;6:27-35.

13. Sanchez PG, D'Ovidio F. Ex-vivo lung perfusion. Curr Opin Organ Transplant 2012;17:490-5.

14. Sanchez PG, Bittle GJ, Burdorf L, et al. State of art: clinical ex vivo lung perfusion: rationale, current status, and future directions. J Heart Lung Transplant 2012;31:339-48.

15. Roman MA, Nair S, Tsui S, et al. Ex vivo lung perfusion: a comprehensive review of the development and exploration of future trends. Transplantation 2013;96:509-18.

16. Andreasson AS, Dark JH, Fisher AJ. Ex-vivo lung perfusion in clinical lung transplantation – state of the art. Eur J Cardiothorac Surg 2014;46:779-88.

17. Van Raemdonck D, Neyrinck A, Cypel M, et al. Ex-vivo lung perfusion. Transpl Int 2015;28:643-56.

18. Sanchez PG, Mackowick KM, Kon ZN. Current state of ex-vivo lung perfusion. Curr Opin Organ Transplant 2016;21:258-66.

19. Cypel M, Keshavjee S. Extracorporeal lung perfusion (ex-vivo lung perfusion). Curr Opin Organ Transplant 2016;21:329-35.

20. Reeb J, Cypel M. Ex vivo lung perfusion. Clin Transplant 2016;30:183-94.

21. D'Cunha HC, Rojas M. Ex-vivo lung perfusion: past, present, and future. ASAIO J 2018;64:135-9.

22. Jacobs S, Rega F, Meyns B. Current preservation technology and future prospects of thoracic organs. Part 2: heart. Curr Opin Organ Transplant 2010;15:156-9.

23. Minasian SM, Galagudza MM, Dmitriev YV, et al. Preservation of the donor heart: from basic science to clinical studies. Interact Cardiovasc Thorac Surg 2015;20:510-9.

24. Van Raemdonck D. Current preservation technology and future prospects of thoracic organs. Part 1: lung. Curr Opin Organ Transplant 2010;15:150-5.

25. Machuca TN, Cypel M, Keshavjee S. Advances in lung preservation. Surg Clin North Am 2013;93:1373-94.

26. Kobashigawa J, Zuckermann A, Macdonald P, et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. J Heart Lung Transplant 2014;33:327-40.

27. Van Raemdonck D, Hartwig MG, Hertz MI, et al. Report of the ISHLT working group on primary lung graft dysfunction Part IV: Prevention and treatment: A 2016 Consensus Group statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2017;36:1121-36.

28. Pirenne J. Time to think out of the (ice) box. Curr Opin Organ Transplant 2010;15:147-9.

29. Proctor E, Parker R. Preservation of isolated heart for 72 hours. Br Med J 1968;4:296-8.

30. Wicomb WN, Cooper DK, Barnard CN. Twenty-four-hour preservation of the pig heart by a portable hypothermic perfusion system. Transplantation 1982;34:246-50.

31. Wicomb WN, Cooper DK, Novitzky D, et al. Cardiac transplantation following storage of the donor heart by a portable hypothermic perfusion system. Ann Thorac Surg 1984;37:243-8.

32. Wicomb WN, Novitzky D, Cooper DK, et al. Forty-eight hours hypothermic perfusion storage of pig and baboon hearts. J Surg Res 1986;40:276-84.

33. Steen S, Paskevicius A, Liao Q, et al. Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. Scand Cardiovasc J 2016;50:193-200.

34. Rosenbaum DH, Peltz M, DiMaio JM, et al. Perfusion preservation versus static preservation for cardiac transplantation: effects on myocardial function and metabolism. J Heart Lung Transplant 2008;27:93-9.

35. Ozeki T, Kwon MH, Gu J, et al. Heart preservation using continuous ex vivo perfusion improves viability and functional recovery. Circ J 2007;71:153-9.

36. Stowe DF, Camara AK, Heisner JS, et al. Low-flow perfusion of guinea pig isolated hearts with 26 degrees C air-saturated Lifor solution for 20 hours preserves function and metabolism. J Heart Lung Transplant 2008;27:1008-15.

37. Michel SG, La Muraglia GM 2nd, Madariaga ML, et al. Preservation of donor hearts using hypothermic oxygenated perfusion. Ann Transplant 2014;19:409-16.

38. Van Caenegem O, Beauloye C, Bertrand L, et al. Hypothermic continuous machine perfusion enables preservation of energy charge and functional recovery of heart grafts in an ex vivo model of donation following circulatory death. Eur J Cardiothorac Surg 2016;49:1348-53.

39. Ou R, Lim YW, Choong JW, et al. Low-flow hypothermic crystalloid perfusion is superior to cold storage during prolonged heart preservation. Transplant Proc 2014;46:3309-13.

40. Hardesty RL, Griffith BP. Autoperfusion of the heart and lungs for preservation during distant procurement. J Thorac Cardiovasc Surg 1987;93:11-8.

41. Chien SF, Diana JN, Oeltgen PR, et al. Functional studies of heart during a 24-hour preservation using a new autoperfusion preparation. J Heart Lung Transplant 1991;10:401-8.

42. Garbade J, Krautz C, Aupperle H, et al. Functional, metabolic, and morphological aspects of continuous, normothermic heart preservation: effects of different preparation and perfusion techniques. Tissue Eng Part C Methods 2009;15:275-83.

43. Hamed A, Tsui S, Huber J, et al. Serum lactate is a highly sensitive and specific predictor of post cardiac transplant outcomes using the Organ Care System. J Heart Lung Transplant 2009;28:S71.

44. Hassanein WH, Zellos L, Tyrrell TA, et al. Continuous perfusion of donor hearts in the beating state extends preservation time and improves recovery of function. J Thorac Cardiovasc Surg 1998;116:821-30.

45. Trahanas JM, Witer LJ, Alghanem F, et al. Achieving 12 Hour normothermic ex situ heart perfusion: an experience of 40 porcine hearts. ASAIO J 2016;62:470-6.

46. Koerner MM, Ghodsizad A, Schulz U, et al. Normothermic ex vivo allograft blood perfusion in clinical heart transplantation. Heart Surg Forum 2014;17:E141-5.

47. Stamp NL, Shah A, Vincent V, et al. Successful heart transplant after ten hours out-of-body time using the TransMedics Organ Care System. Heart Lung Circ 2015;24:611-3.

48. Chan JL, Kobashigawa JA, Reich HJ, et al. Intermediate outcomes with ex-vivo allograft perfusion for heart transplantation. J Heart Lung Transplant 2017;36:258-63.

49. McCurry K, Jeevanandam V, Mihaljevic T, et al. Prospective multi-center safety and effectiveness evaluation of the organ care system device for cardiac use (PROCEED). J Heart Lung Transplant 2008;27:S166.

50. Tenderich G, El-Banayosy A, Rosengard B, et al. Prospective multi-center European trial to evaluate the safety and performance of the organ care system for heart transplants (PROTECT). J Heart Lung Transplant 2007;26:S64.

51. Ardehali A, Esmaillian F, Deng M, et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. Lancet 2015;385:2577-84.

52. Randomized Study of Organ Care System Cardiac for Preservation of Donated Hearts for Eventual Transplantation (PROCEEDII). Available online: https://clinicaltrials.gov/show/NCT00855712, accessed December 28, 2017.

53. Freed DH, White CW. Donor heart preservation: straight up, or on the rocks? Lancet 2015;385:2552-4.

54. Leprince P, Popov AF, Simon AR, et al. Ex vivo perfusion of the heart with the use of the Organ Care System. Eur J Cardiothorac Surg 2016;49:1318-20.

55. Thabut G, Mal H, Cerrina J, et al. Graft ischemic time and outcome of lung transplantation: a multicenter analysis. Am J Respir Crit Care Med 2005;171:786-91.

56. Grimm JC, Valero V 3rd, Kilic A, et al. Association between prolonged graft ischemia and primary graft failure or survival following lung transplantation. JAMA Surg 2015;150:547-53.

57. Yeung JC, Krueger T, Yasufuku K, et al. Outcomes after transplantation of lungs preserved for more than 12 h: a retrospective study. Lancet Respir Med 2017;5:119-24.

58. Jirsch DW, Fisk RL, Couves CM. Ex vivo evaluation of stored lungs. Ann Thorac Surg 1970;10:163-8.

59. Steen S, Sjoberg T, Pierre L, et al. Transplantation of lungs from a non-heart-beating donor. Lancet 2001;357:825-9.

60. Steen S, Liao Q, Wierup PN, et al. Transplantation of lungs from non-heart-beating donors after functional assessment ex vivo. Ann Thorac Surg 2003;76:244-52.

61. Cypel M, Yeung JC, Hirayama S, et al. Technique for prolonged normothermic ex vivo lung perfusion. J Heart Lung Transplant 2008;27:1319-25.

62. Cypel M, Rubacha M, Yeung J, et al. Normothermic ex vivo perfusion prevents lung injury compared to extended cold preservation for transplantation. Am J Transplant 2009;9:2262-9.

63. International Randomized Study of the TransMedics Organ Care System (OCS Lung) for Lung Preservation and Transplantation (INSPIRE). Available online: http://clinicaltrials.gov/ct2/show/NCT01630434?term=Inspire&rank=7, accessed December 28, 2017.

64. Warnecke G, Van Raemdonck D, Kukreja J, et al. Mid and long-term clinical results of OCS Lung Inspire International trial. J Heart Lung Transplant 2016;35:S15-6.

65. Hsin MK, Iskender I, Nakajima D, at al. Extension of donor lung preservation with hypothermic storage after normothermic ex vivo lung perfusion. J Heart Lung Transplant 2016;35:130-6.

66. Slama A, Schillab L, Barta M, et al. Standard donor lung procurement with normothermic ex vivo lung perfusion: A prospective randomized clinical trial. J Heart Lung Transplant 2017;36:744-53.

67. Martens A, Van Raemdonck DE, Smits J, et al. A retrospective database analysis to evaluate the potential of ex vivo lung perfusion to recruit declined lung donors. Transpl Int 2017;30:1002-10.

68. García Sáez D, Zych B, Sabashnikov A, et al. Evaluation of the organ care system in heart transplantation with an adverse donor/recipient profile. Ann Thorac Surg

2014;98:2099-105.

69. International EXPAND Heart Pivotal Trial (EXPANDHeart) Available online: http://clinicaltrials.gov/ct2/show/NCT02323321?term=Inspire&rank=7, accessed December 28, 2017.

70. Ali AA, White P, Xiang B, et al. Hearts from DCD donors display acceptable biventricular function after heart transplantation in pigs. Am J Transplant 2011;11:1621-32.

71. Colah S, Freed DH, Mundt P, et al. Ex vivo perfusion of the swine heart as a method for pre-transplant assessment. Perfusion 2012;27:408-13.

72. White CW, Ali A, Hasanally D, et al. A cardioprotective preservation strategy employing ex vivo heart perfusion facilitates successful transplant of donor hearts after cardiocirculatory death. J Heart Lung Transplant 2013;32:734-43.

73. Iyer A, Gao L, Doyle A, et al. Normothermic ex vivo perfusion provides superior organ preservation and enables viability assessment of hearts from DCD donors. Am J Transplant 2015;15:371-80.

74. García Sáez D, Elbetanony A, Lezberg P, et al. Ex vivo heart perfusion after cardiocirculatory death; a porcine model. J Surg Res 2015;195:311-4.

75. White CW, Lillico R, Sandha J, et al. Physiologic changes in the heart following cessation of mechanical ventilation in a porcine model of donation after circulatory death: implications for cardiac transplantation. Am J Transplant 2016;16:783-93.

76. Ali A, White P, Dhital K, et al. Cardiac recovery in a human non-heart-beating donor after extracorporeal perfusion: source for human heart donation? J Heart Lung Transplant 2009;28:290-3.

77. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. Lancet 2015;385:2585-91.

78. Macdonald P, Verran D, O'Leary M, et al. Heart transplantation from donation after circulatory death donors. Transplantation 2015;99:1101-2.

79. Dhital KK, Chew HC, Macdonald PS. Donation after circulatory death heart transplantation. Curr Opin Organ Transplant 2017;22:189-97.

80. Messer S, Large S. Resuscitating heart transplantation: the donation after circulatory determined death donor. Eur J Cardiothorac Surg 2016;49:1-4.

81. Messer SJ, Axell RG, Colah S, et al. Functional assessment and transplantation of the donor heart after circulatory death. J Heart Lung Transplant 2016;35:1443-52.

82. Tsui SSL, Oniscu GC. Extending normothermic regional perfusion to the thorax in donors after circulatory death. Curr Opin Organ Transplant 2017;22:245-50.

83. Ingemansson R, Eyjolfsson A, Mared L, et al. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. Ann Thorac Surg 2009;87:255-60.

84. Cypel M, Yeung J, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. N Engl J Med 2011;364:1431-40.

85. Lindstedt S, Hlebowicz J, Koul B, et al. Comparative outcome of double lung transplantation using conventional donor lungs and non-acceptable donor lungs reconditioned ex vivo. Interact Cardiovasc Thorac Surg 2011;12:162-5.

86. Zych B, Popov AF, Stavri G, et al. Early outcomes of bilateral sequential single lung transplantation after ex-vivo lung evaluation and reconditioning. J Heart Lung Transplant 2012;31:274-81.

87. Aigner C, Slama A, Hötzenecker K, et al. Clinical ex vivo lung perfusion – pushing the limits. Am J Transplant 2012;12:1839-47.

88. Cypel M, Yeung JC, Machuca T, et al. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. J Thorac Cardiovasc Surg 2012;144:1200-6.

89. Wallinder A, Ricksten SE, Hansson C, et al. Transplantation of initially rejected donor lungs after ex vivo lung perfusion. J Thorac Cardiovasc Surg 2012;144:1222-8.

90. Warnecke G, Moradiellos J, Tudorache I, et al. Normothermic perfusion of donor lungs for preservation and assessment with the Organ Care System Lung before bilateral transplantation: a pilot study of 12 patients. Lancet 2012;380:1851-8.

91. Wallinder A, Ricksten SE, Silverborn M, et al. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study. Eur J Cardiothorac Surg 2014:45:40-4.

92. Dark JH, Karamanou D, Clark S, et al. Successful transplantation of unusable donor lungs using ex-vivo lung perfusion: the Newcastle experience (abstract 323). J Heart Lung Transplant 2012;31:S115.

93. Valenza F, Rosso L, Coppola S, et al. Ex vivo lung perfusion to improve donor lung function and increase the number of organs available for transplantation. Transpl Int 2014;27:553-61.

94. Henriksen IS, Møller-Sørensen H, Møller CH, et al. First Danish experience with ex vivo lung perfusion of donor lungs before transplantation. Dan Med J 2014;61:A4809.

95. Sage E, Mussot S, Trebbia G, et al. Lung transplantation from initially rejected donors after ex vivo lung

reconditioning: the French experience. Eur J Cardiothorac Surg 2014;46:794-9.

96. Boffini M, Ricci D, Bonato R, et al. Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with ex vivo lung perfusion. Eur J Cardiothorac Surg 2014;46:789-93.

97. Fildes JE, Archer LD, Blaikley J, et al. Clinical outcome of patients transplanted with marginal donor lungs via ex vivo lung perfusion compared to standard lung transplantation. Transplantation 2015;99:1078-83.

98. Wallinder A, Riise GC, Ricksten SE. Transplantation after ex vivo lung perfusion: A midterm follow-up. J Heart Lung Transplant 2016;35:1303-10.

99. Zeriouh M, Sabashnikov A, Mohite PN, et al. Utilization of the organ care system for bilateral lung transplantation: preliminary results of a comparative study. Interact Cardiovasc Thorac Surg 2016;23:351-7.

100. Cypel M, Keshavjee S. Extending the donor pool: rehabilitation of poor organs. Thorac Surg Clin 2015;25:27-33.

101. Tikkanen JM, Cypel M, Machuca TN, et al. Functional outcomes and quality of life after normothermic ex vivo lung perfusion lung transplantation. J Heart Lung Transplant 2015;34:547-56.

102. A study of donor lung perfusion in lung transplantation in United Kingdom. Available online: http://www.controlled-trials.com/ISRCTN44922411, accessed December 28, 2017.

103. Fisher A, Andreasson A, Chrysos A, et al. An observational study of Donor Ex Vivo Lung Perfusion in UK lung transplantation: DEVELOP-UK. Health Technol Assess 2016;20:1-276.

104. Novel Lung Trial: Normothermic ex vivo lung perfusion (Evlp) as an assessment of extended/marginal donor lungs. Available online: http://clinicaltrials.gov/ct2/show/NCT01365429?term=NOVEL&rank=406, accessed December 28, 2017.

105. Sanchez PG, Davis RD, D'Ovidio F, et al. The NOVEL lung trial one-year outcomes (abstract 181). J Heart Lung Transplant 2014;33:S71-2.

106. International EXPAND Lung Pivotal Trial (EXPANDLung). Available online: http://clinicaltrials.gov/ct2/show/NCT01963780?term=Expand&rank=42, accessed December 28, 2017.

107. Van Raemdonck D, Warnecke G, Kukreja J, et al. The EXPAND Lung International Trial to Evaluate the Safety and Effectiveness of the Portable Organ Care System (OCS™) Lung for Recruiting, Preserving and Assessing Expanded Criteria Donor Lungs for Transplantation

(abstract 26). J Heart Lung Transplant 2014;33:S17-8.

108. Loor G, Warnecke G, Smith J, et al. The OCS Lung Expand international trial interim results. J Heart Lung Transplant 2016;35:S68-9.

109. Extending Preservation and Assessment Time of Donor Lungs Using the Toronto EVLP System™ at a Dedicated EVLP Facility. Available online: https://clinicaltrials.gov/ct2/show/NCT02234128, accessed December 28, 2017.

110. Cypel M, Levvey B, Van Raemdonck D, et al. International Society for Heart and Lung Transplantation donation after circulatory death registry report. J Heart Lung Transplant 2015;34:1278-82.

111. Krutsinger D, Reed RM, Blevins A, et al. Lung transplantation from donation after cardiocirculatory death: a systematic review and meta-analysis. J Heart Lung Transplant 2015;34:675-84.

112. Machuca TN, Mercier O, Collaud S, et al. Lung transplantation with donation after circulatory determination of death donors and the impact of ex vivo lung perfusion. Am J Transplant 2015;15:993-1002.

113. Ruttens D, Martens A, Ordies S, et al. Short- and long-term outcome after lung transplantation from circulatory-dead donors: a single-center experience. Transplantation 2017;101:2691-4.

114. Mohite PN, Sabashnikov A, García Sáez D, et al. Utilization of the Organ Care System Lung for the assessment of lungs from a donor after cardiac death (DCD) before bilateral transplantation. Perfusion 2015;30:427-30.

115. Gomez-de-Antonio D, Campo-Cañaveral JL, Crowley S, et al. Clinical lung transplantation from uncontrolled non-heart-beating donors revisited. J Heart Lung Transplant 2012;31:349-53.

116. Moradiellos FJ, Naranjo JM, Cordoba M, et al. Clinical lung transplantation after ex vivo evaluation of uncontrolled non heart-beating donors lungs: initial experience. J Heart Lung Transplant 2011;30:S38.

117. Valenza F, Citerio G, Palleschi A, et al. Successful transplantation of lungs from an uncontrolled donor after circulatory death preserved In situ by alveolar recruitment maneuvers and assessed by ex vivo lung perfusion. Am J Transplant 2016;16:1312-8.

118. Bozso S, Vasanthan V, Luc JG, et al. Lung transplantation from donors after circulatory death using portable ex vivo lung perfusion. Can Respir J 2015;22:47-51.

119. Levin K, Kotecha S, Westall G, et al. How can we improve the quality of transplantable lungs? Expert Rev Respir Med 2016:1-7. [Epub ahead of print].

120. Warnecke G. Ex vivo treatment. Transplantation

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2016;100:1400-1.

121. Ordies S, Neyrinck A, Van Raemdonck D. Can we make recovered donor lungs look brand-new again? Eur J Cardiothorac Surg 2017;52:178-9.

122. Andreasson A, Karamanou DM, Perry JD, et al. The effect of ex vivo lung perfusion on microbial load in human donor lungs. J Heart Lung Transplant 2014;33:910-6.

123. Nakajima D, Cypel M, Bonato R, et al. Ex vivo perfusion treatment of infection in human donor lungs. Am J Transplant 2016;16:1229-37.

124. Meers CM, Wauters S, Verbeken E, et al. Preemptive therapy with steroids but not macrolides improves gas exchange in caustic-injured donor lungs. J Surg Res 2011;170:e141-8.

125. Yamada Y, Iskender I, Arni S, et al. Ex vivo treatment with inhaled N-acetylcysteine in porcine lung transplantation. J Surg Res 2017;218:341-7.

126. Lin H, Chen M, Tian F, et al. α1-anti-trypsine improves function of porcine donor lungs during ex-vivo lung perfusion. J Heart Lung Transplant 2017. [Epub ahead of print].

127. Cosgun T, Iskender I, Yamada Y, et al. Ex vivo administration of trimetazidine improves post-transplant lung function in pig model. Eur J Cardiothorac Surg 2017;52:171-7.

128. Charles EJ, Mehaffey JH, Sharma AK, et al. Lungs donated after circulatory death and prolonged warm ischemia are transplanted successfully after enhanced ex vivo lung perfusion using adenosine A2B receptor antagonism. J Thorac Cardiovasc Surg 2017;154:1811-20.

129. Wang X, Wang Y, Parapanov R, et al. Pharmacological reconditioning of marginal donor rat lungs using inhibitors of peroxynitrite and poly (ADP-ribose) polymerase during ex vivo lung perfusion. Transplantation 2016;100:1465-73.

130. Inci I, Zhai W, Arni S, et al. Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors. J Heart Lung Transplant 2007;26:1054-60.

131. Cypel M, Liu M, Rubacha M, et al. Functional repair of human donor lungs by IL-10 gene therapy. Sci Transl Med 2009;1:4ra9.

132. Yeung JC, Cypel M, Keshavjee S. Ex-vivo lung perfusion: the model for the organ reconditioning hub. Curr Opin Organ Transplant 2017;22:287-9.

133. Iyer A, Gao L, Doyle A, et al. Increasing the tolerance of DCD hearts to warm ischemia by pharmacological postconditioning. Am J Transplant 2014;14:1744-52.

134. White CW, Hasanally D, Mundt P, et al. A whole

blood-based perfusate provides superior preservation of myocardial function during ex vivo heart perfusion. J Heart Lung Transplant 2015;34:113-21.

135. Inci I, Ampollini L, Arni S, et al. Ex vivo reconditioning of marginal donor lung injured by acid aspiration. J Heart Lung Transplant 2008;27:1229-36.

136. Martens A, Ordies S, Vanaudenaerde BM, et al. A porcine ex vivo lung perfusion model with maximal argon exposure to attenuate ischemia-reperfusion injury. Med Gas Res 2017;7:28-36.

137. Hijiya K, Chen-Yoshikawa TF, Kondo T, et al. Bronchodilator inhalation during ex vivo lung perfusion improves posttransplant graft function after warm ischemia. Ann Thorac Surg 2017;103:447-53.

138. Nilsson T, Gielis JF, Slama A, et al. Comparison of two strategies for ex vivo lung perfusion. J Heart Lung Transplant 2017. [Epub ahead of print].

139. Loor G, Howard BT, Spratt JR, et al. Prolonged EVLP using OCS Lung: cellular and acellular perfusates. Transplantation 2017;101:2303-11.

140. Becker S, Steinmeyer J, Avsar M, et al. Evaluating acellular versus cellular perfusate composition during prolonged ex vivo lung perfusion after initial cold ischaemia for 24 hours. Transpl Int 2016;29:88-97.

141. Roman M, Gjorgjimajkoska O, Neil D, et al. Comparison between cellular and acellular perfusates for ex vivo lung perfusion in a porcine model. J Heart Lung Transplant 2015;34:978-87.

142. Linacre V, Cypel M, Machuca T, et al. Importance of left atrial pressure during ex vivo lung perfusion. J Heart Lung Transplant 2016;35:808-14.

143. Aboelnazar NS, Himmat S, Hatami S, et al. Negative pressure ventilation decreases inflammation and lung edema during normothermic ex-vivo lung perfusion. J Heart Lung Transplant 2018;37:520-30.

144. Luc JGY, Aboelnazar NS, Himmat S, et al. A leukocyte filter does not provide further benefit during ex vivo lung perfusion. ASAIO J 2017;63:672-8.

145. Kakishita T, Oto T, Hori S, et al. Suppression of inflammatory cytokines during ex vivo lung perfusion with an adsorbent membrane. Ann Thorac Surg 2010;89:1773-9.

146. Iskender I, Cosgun T, Arni S, et al. Cytokine filtration modulates pulmonary metabolism and edema formation during ex vivo lung perfusion. J Heart Lung Transplant 2017. [Epub ahead of print].

147. Noda K, Tane S, Haam SJ, et al. Optimal ex vivo lung perfusion techniques with oxygenated perfusate. J Heart

Lung Transplant 2017;36:466-74.

148. Nilsson T, Hansson C, Wallinder A, et al. Hemofiltration in ex vivo lung perfusion-a study in experimentally induced pulmonary edema. J Thorac Cardiovasc Surg 2016;151:570-5.e1.

149. Andreasson ASI, Borthwick LA, Gillespie C, et al. The role of interleukin-1β as a predictive biomarker and potential therapeutic target during clinical ex vivo lung perfusion. J Heart Lung Transplant 2017;36:985-95.

150. Machuca TN, Cypel M, Zhao Y, et al. The role of the endothelin-1 pathway as a biomarker for donor lung assessment in clinical ex vivo lung perfusion. J Heart Lung Transplant 2015;34:849-57.

151. Hashimoto K, Cypel M, Kim H, et al. Soluble adhesion molecules during ex vivo lung perfusion are associated with posttransplant primary graft dysfunction. Am J Transplant 2017;17:1396-404.

152. Hsin MK, Zamel R, Cypel M, et al. Metabolic profile of ex vivo lung perfusion yields biomarkers for lung transplant outcomes. Ann Surg 2018;267:196-7.

153. Sage E, De Wolf J, Puyo P, et al. Real-time computed tomography highlights pulmonary parenchymal evolution during ex vivo lung reconditioning. Ann Thorac Surg 2017;103:e535-7.

154. Verleden SE, Martens A, Ordies S, et al. Radiological analysis of unused donor lungs: a tool to improve donor acceptance for transplantation? Am J Transplant 2017;17:1912-21.

155. Sanchez PG, Iacono AT, Rajagopal K, et al. Successful lung salvage by ex vivo reconditioning of neurogenic pulmonary edema: case report. Transplant Proc 2014;46:2453-5.

156. Inci I, Yamada Y, Hillinger S, et al. Successful lung transplantation after donor lung reconditioning with urokinase in ex vivo lung perfusion system. Ann Thorac Surg 2014;98:1837-8.

157. Machuca TN, Hsin MK, Ott HC, et al. Injury-specific ex vivo treatment of the donor lung: pulmonary thrombolysis followed by successful lung transplantation. Am J Respir Crit Care Med 2013;188:878-80.

158. Stone JP, Critchley WR, Major T, et al. Altered immunogenicity of donor lungs via removal of passenger leukocytes using ex vivo lung perfusion. Am J Transplant 2016;16:33-43.

159. Noda K, Tane S, Haam SJ, et al. Targeting circulating leukocytes and pyroptosis during ex vivo lung perfusion improves lung preservation. Transplantation 2017;101:2841-9.

160. Van Raemdonck D, Neyrinck A, Rega F, et al. Machine perfusion in organ transplantation: a tool for ex-vivo graft conditioning with mesenchymal stem cells? Curr Opin Organ Transplant 2013;18:24-33.

161. English K, Mahon BP. Allogeneic mesenchymal stem cells: agents of immune modulation. J Cell Biochem 2011;112:1963-8.

162. Roemeling-van Rhijn M, Weimar W, Hoogduijn MJ. Mesenchymal stem cells: application for solid-organ transplantation. Curr Opin Organ Transplant 2012;17:55-62.

163. Hoogduijn MJ, Popp FC, Grohnert A, et al. Advancement of mesenchymal stem cell therapy in solid organ transplantation (MISOT). Transplantation 2010;90:124-6.

164. Lee JW, Fang X, Krasnodembskaya A, et al. Concise review: mesenchymal stem cells for acute lung injury: role of paracrine soluble factors. Stem Cells 2011;29:913-9.

165. Matthay MA, Pati S, Lee JW. Concise Review: Mesenchymal stem (stromal) cells: biology and preclinical evidence for therapeutic potential for organ dysfunction following trauma or sepsis. Stem Cells 2017;35:316-24.

166. Stone ML, Zhao Y, Smith JR, et al. Mesenchymal stromal cell-derived extracellular vesicles attenuate lung ischemia-reperfusion inhury and enhance reconditioning of donor lungs after circulatory death. Respir Res 2017;18:212.

167. Mordant P, Nakajima D, Kalaf R, et al. Mesenchymal stem cell treatment is associated with decreased perfusate concentration of interleukin-8 during ex vivo perfusion of donor lungs after 18-hour preservation. J Heart Lung Transplant 2016;35:1245-54.

168. Martens A, Ordies S, Vanaudenaerde BM, et al. Immunoregulatory effects of multipotent adult progenitor cells in a porcine ex vivo lung perfusion model. Stem Cell Res Ther 2017;8:159.

169. Casiraghi F, Noris M, Remuzzi G. Immunomodulatory effect of mesenchymal stromal cells in solid organ transplantation. Curr Opin Organ Transplant 2010;15:731-7.

170. Franquesa M, Hoogduijn MJ, Baan CC. The impact of mesenchymal stem cell therapy in transplant rejection and tolerance. Curr Opin Organ Transplant 2012;17:355-61.

171. Casiraghi F, Perico N, Remuzzi G. Mesenchymal stromal cells to promote solid organ transplantation tolerance. Curr Opin Organ Transplant 2013;18:51-8.

Donors after cardiocirculatory death and lung transplantation

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Abstract: The number of patients actively awaiting lung transplantation (LTx) is more than the number of suitable donor lungs. The percentage of lung retrieval rate is lower when compared to other solid organs. The use of lungs from donation after cardiocirculatory death (DCD) donors is one of the options to avoid organ shortage in LTx. After extensive experimental research, clinical application of DCD donation is becoming wider. The results from most of the centers show at least equal survival rate compared to donors from brain death. This review paper will summarize experimental background and clinical experience from DCD donors.

Keywords: Donation after cardiocirculatory death (DCD); lung transplantation (LTx); lung; survival

Introduction

The number of patients actively awaiting lung transplantation (LTx) is more than the number of suitable donor lungs. The percentage of lung retrieval rate is lower when compared to other solid organs. Brain death itself leads to hemodynamic, metabolic and neuroendocrine abnormalities resulting in so-called neurogenic pulmonary edema (1,2). This initial insult in combination with possible airway aspiration, respiratory tract infection, atelectasis and pulmonary contusion, may all contribute to lung damage before harvest (1).

The use of lungs from donation after cardiocirculatory death (DCD) donors is one of the options to avoid organ shortage in LTx (3-16). The number of lung transplants performed from DCD donors is increasing. A recent International Society for Heart and Lung Transplantation (ISHLT) DCD Registry Report included 306 recipients among ten centers worldwide (12). Several centers published their experience, most of them with excellent or at least equal results compared to brain-dead donors (5,14,17-28).

The first successful attempt of human LTx (29), and the first long-term successful human LTx (30) utilized DCD donors. Thereafter the concept of brain death and organ donation after brain death (DBD) became more widely accepted (11) and because of this DCD was largely abandoned.

Proof of concept and experimental background

Thomas M. Egan reintroduced the concept of LTx from DCD donors in 1991 following a series of dog experiments (31). He showed that the lung may remain viable for a certain period after death as a result of the oxygen reserve present in the alveoli.

To investigate the hypothesis that lungs may be suitable for transplant even if explanted at substantial interval after death, Egan *et al.*, used a canine single left lung transplant model (31). They retrieved left lungs at 1, 2, or 4 h after death from non-ventilated donors. Following the transplantation, they ligated the contralateral pulmonary artery and bronchus 1 h after transplantation to force the recipients survive solely on the transplanted lung retrieved from DCD donor. All recipients of lungs retrieved 1 h after death survived the 8-h observation period with good gas exchange. Two of the five recipients of 2-h cadaver lungs survived with good gas exchange, whereas gas exchange and survival were poor in recipients of lungs retrieved 4 h after death (31).

In order to find out the time course of pulmonary cell death after circulatory arrest D'Armini *et al.* from Egan's group used trypan blue dye exclusion to quantitate lung cell death at postmortem intervals in rats. Postmortem mechanical ventilation with oxygen appeared to delay lung death in the rat DCD model (32).

To determine postmortem adenine nucleotide tissue levels in the lung and their relationship to lung viability D'Armini *et al.* showed that by 4 h after death, the viability was 85% in the O_2-ventilated cadaver rat lungs, significantly higher than in the N_2-ventilated (43%) and in the non-ventilated (48%) lungs (33).

In a dog model, Ulicny *et al.* retrieved lungs 4 h after death from ventilated DCD donors (34). Four of six recipients of oxygen-ventilated cadaver lungs survived 8 h with good gas exchange whereas two of six recipients of non-ventilated lungs survived with poor gas exchange. With additional canine studies, they demonstrated benefit of flushing lungs with solution containing a free radical scavenger, dimethylthiourea (35,36). Donor lung ventilation with alveolar gas (20% O_2, 5% CO_2, balanced N_2) during 4-h warm ischemic time (WIT) did not result in improved lung function (37). DCD donors ventilated with 100% O_2 prior to organ retrieval showed superior pulmonary function after transplantation compared with lungs grafts ventilated with alveolar gas (37).

Rega *et al.* showed that NAC administered before or shortly after death attenuated early ischemia-reperfusion injury via up-regulation of glutathione (38).

In a pig model, after 1 h *in situ* WIT the lungs were either topically cooled or ventilated for 3 h. Topically cooled lungs showed better function compared to ventilation-only group (39).

In a pig DCD model, donors with increasing time intervals of 1, 2, and 3 h and donors from heart-beating animals were assessed in *ex vivo* perfusion system. They found a strong correlation between the increase of IL-1beta concentration and the increase in pulmonary vascular resistance, mean airway pressure, and wet-to-dry weight ratio. They concluded that IL-1 beta in bronchial lavage fluid might be a useful, non-invasive marker that can predict the viability of the pulmonary graft from the DCD donors (40).

In dog model, Dougherty *et al.* were able to reduce the core temperature to 2 to 7 ℃ when one lung was ventilated with air delivered at subzero temperature (–10 to –15 ℃) during 1 h (41). However, recipients did not survive on this lung alone because of the development capillary leak with edema as a result of the freezing damage (41). In a dog model Watanabe *et al.* were successful in transplanting DCD donor lungs that were cooled for 2 h by filling one hemithorax with cold air (42). Steen *et al.* in a pig DCD model with open chest, cooled donor lungs with saline slush placed in both pleural cavities (43). Lung core temperature decreased to less than 10 ℃ within 40 minutes and topical cooling was continued for 6 h. All six recipients survived for 24 h on the transplanted left lung with the exclusion of the right native lung (43). In order to create a clinically relevant situation, Steen's group cooled the lungs topically *in situ* by continuous infusion of cold preservation solution via two intrapleural drains inserted via two small intercostal incisions (44).

The efficacy of partial liquid ventilation (PLV) with perfluorocarbon in lung protection during hypotension and cardiac arrest has been studied by Yoshida *et al.* (45). Using rabbit lungs, they maintained hypotension at <50 mmHg for 1 h followed by 2-h cardiac arrest. Histologic evaluation after perfusion of the preservation solution revealed that alveolar structure was damaged significantly less and cell infiltration was milder in the PLV groups than in the control group (45). Tissue IL-8 in the PLV groups remained at baseline concentrations during the study period. They concluded that PLV suppresses lung injury when compared with gas-controlled ventilation (45).

Okazaki *et al.* evaluated the optimal time for post-mortem heparinization in canine LTx from DCD donors (46). The cadaver donors were assigned randomly to one of five study groups. They reported that the optimal time for post-mortem heparinization in LTx from DCD donors was approximately 30 minutes after cardiac arrest (46).

Using *ex vivo* lung perfusion (EVLP) method we demonstrated that administration of urokinase during EVLP after 3 h of warm ischemia improved lung function by dissolving microthrombi with its fibrinolytic action (47).

We also investigated the impact of topical cooling solution and prediction of graft function from DCD donors (48). We found that topical cooling with Perfadex after 3 h of death resulted in improved graft function compared to saline group. However, graft parameters were comparable between saline and Perfadex groups after 1 h of warm ischemia (48).

To assess the surfactant alterations in DCD donor lungs (49) we showed that surfactant function decreases with increased WITs. This was proven by significantly different adsorption and surface tension in DCD groups compared with heart-beating donor (HBD) group (49).

Table 1 The Maastricht categories of DCD (53)

Category I	Dead on arrival at hospital
Category II	Death with Unsuccessful resuscitation
Category III	Awaiting cardiac death
Category IV	Cardiac arrest while brain dead

Table 2 The modified Maastricht classification of DCD (3)

Categories	Outcomes
Category I	Found dead
Uncontrolled*	
IA: out-of-hospital	
IB: in-hospital	
Category II	Witnessed cardiac arrest
Uncontrolled**	
IIA: out-of-hospital	
IIB: in-hospital	
Category III	–
Controlled	
Withdrawal of life sustaining therapy, planned withdrawal of life-sustaining therapy; expected cardiac arrest	
Category IV	–
Controlled***	
Cardiac arrest while brain-dead	
Category V	–
Euthanasia and subsequent organ donation	

*, sudden unexpected CA without any attempt of resuscitation by a life-medical team; WIT to be considered according to National life-recommendations in place; reference to in- or out-of-hospital life setting; **, sudden unexpected irreversible CA with unsuccessful resuscitation life-by a life-medical team; reference to in- or out-of-hospital life setting; ***, sudden cardiac arrest after brain death diagnosis during donor life-management but prior to planned organ recovery. CA, circulatory arrest.

In another study, we tested whether an injured lung graft from a category-3 DCD donor could be reconditioned with EVLP by intra-bronchial diluted surfactant lavage prior to transplantation (50). Our data demonstrated the feasibility of reconditioning and transplantation of an acutely damaged lung graft due to aspiration from a category-3 DCD donor (50).

Martens *et al.* demonstrated that warm ischemic injury in DCD donation could be attenuated by steroids when given prior to warm ischemia and during EVLP (51).

In a mice model, Huerter *et al.* demonstrated that adenosine A2B receptor (A2BR) antagonism attenuated lung ischemia reperfusion injury and augments reconditioning of DCD lungs by EVLP (52). The protective effects of A2BR antagonist (ATL802) might involve targeting A2BRs on alveolar epithelial cells to prevent IL-8 production. A2BR might be a novel therapeutic target for mitigating ischemia reperfusion injury to increase the success of LTx (52).

Clinical experience with DCD donors

Definition and categories

DCD donors are defined as when organs are removed from donors after cardiac arrest (1). According to Maastricht classification, there are four types of DCD donors (*Table 1*) (53). The first two categories are uncontrolled DCD (uDCD) donors. An uDCD donor may occur when a person dies unexpectedly. In these cases, the deceased person may become a potential donor if his or her organs can be adequately preserved inside the cadaver before organ retrieval and if the consent for the retrieval of organs can be obtained from the relatives (1). The exact length of the postmortem WIT is often not known. As organ function in these donors cannot be assessed before death, viability should be properly evaluated afterwards before organ transplantation to reduce the risk of primary non-function (1,6,8).

In the controlled DCD (cDCD) donors (categories III and IV), pulmonary graft assessment can be made after informed consent in the hours before withdrawal of life support in the same way as practiced in the HBD (chest X-ray, oxygenation, bronchoscopy) (1). The warm ischemic period of the graft is limited to 10 to 15 minutes after death certification if withdrawal of life support is executed in the operating room. Lungs can be inspected *in situ*, and preserved in the standard way (1). Recently, modified Maastricht classification of DCD has been published (*Table 2*) (3).

Definitions of WIT

The length of tolerable WIT for DCD donor lungs remains debatable; however, the majority of experimental data suggest that lungs remain viable for at least 60 to 90 min

after circulatory arrest (1,2,54,55).

The clinical limit and most relevant definition of WIT for DCD donor lungs is still debatable (54). It has have been recommended to record prospectively post-withdrawal and postmortem DCD donor hemodynamics and oximetry in order to determine the range, pattern, and potential clinical relevance to DCD clinical lung transplant outcomes (54). Levvey *et al.* from Alfred Hospital, Melbourne recommended different definitions of WIT including the timing of withdrawal, systolic blood pressure (sBP) less than 50 mmHg, initiation of ventilation or the onset of pulmonary arterial flush (54). They suggested WIT definition starting when sBP <50 mmHg and finishing with cold arterial flush (54). This group emphasized the importance of prospectively collecting data on all potential DCD lung donors and to correlate these with clinical outcomes (54). Definitions that start with sBP <50 mmHg represent the start of serious hemodynamic compromise

and might better correlate with clinically significant loss of organ perfusion (54).

In order to standardize the definitions around important times in DCD donation process, ISHLT DCD Working Group recommended the following times points and intervals (12). *Table 3* and *Figure 1* show schematic presentation of the time points and intervals recommended by ISHLT DCD Working Group (12).

The intervals of times in *Figure 1* were defined as: T0 to T2 (interval 1), T0 to T3 (interval 2), T0 to T5 (interval 3) and T2 to T5 (interval 4) (12).

Donor selection criteria

cDCD donors

For cDCD donor selection, most of the centers apply internationally agreed DBD donor criteria (*Table 4*) (7).

Extended criteria donors such as age >65 years, smoking history of >20 pack/years, ICU stay >5 days, and abnormal chest X-ray are accepted in some programs (7). Significant aspiration and a PaO_2/FiO_2 <300 mmHg are generally not accepted for DCD donation (7,15).

Important issues in clinical DCD practice
(I)   Pre-mortem heparin use;
(II)   Pre-mortem bronchoscopy;
(III)   Placement of nasogastric tube;
(IV)   Stand-off period;
(V)   Length of agonal phase;

Table 3 Time points suggested by ISHLT DCD Working Group (12)

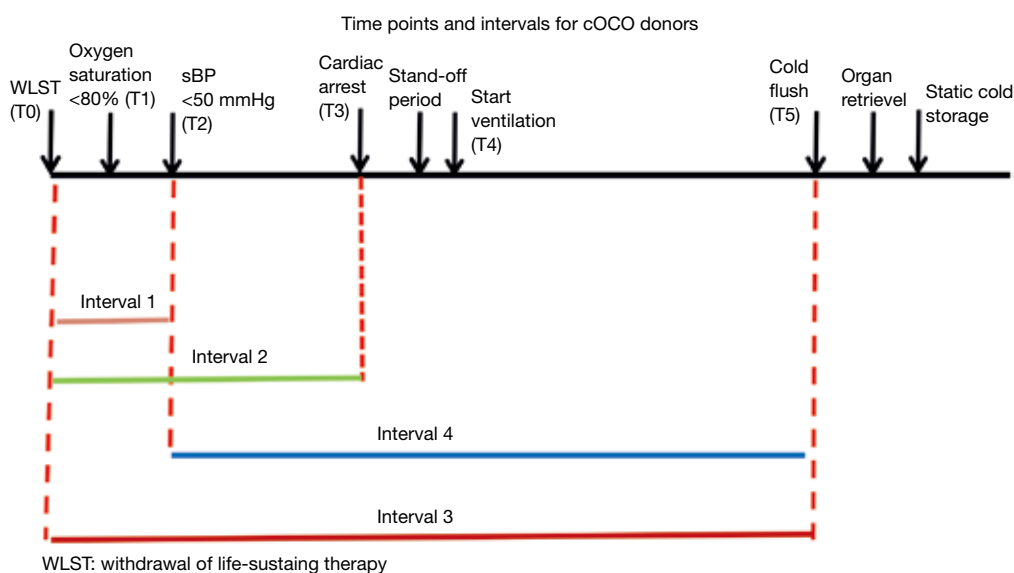|	
T0	Withdrawal of life-sustaining therapies or euthanasia
T1	Oxygen saturation <80%
T2	Systolic blood pressure <50 mmHg
T3	Cessation of cardiac output/asystole
T4	Resumed lung inflation/ventilation
T5	Start of pulmonary flush

Figure 1 Schematic presentation of the time points and intervals for cDCD donors recommended by ISHLT DCD Working Group (14).

Table 4 DCD donor criteria (7)

Age	<65 years
Smoking	<20 pack/years
CXR	Clear
Mechanical ventilation	<5 days
Blood transfusion	<5 units RBC
Oxygenation	PaO_2 >40 kPa

(VI)   Withdrawal of tracheal tube;
(VII)   Maximal length of initial warm ischemic period;
(VIII) Timing of re-ventilation;
(IX)   Selective use of EVLP.

Pre-mortem interventions in a patient who is a potential DCD donor vary widely among the centers due to ethical considerations (27,56-58).

In a patient who is not declared a donor until death, appropriate and maximum treatment of the patient should be continued (7). The other issue is to protect the organ for good outcomes after transplantation. Lung protective ventilation that reduces lung injury (i.e., a tidal volume of 6–8 mL/kg ideal body weight, with PEEP of 8 cmH$_2$O, frequent suctioning) is recommended (7). A pre-mortem bronchoscopy is generally performed among the centers (17,21,23,59,60) to assess the airways and the placement of a nasogastric tube to prevent aspiration of gastric contents (17,59). The airways of a potential DCD donor might be protected from aspiration by omitting extubation; on the other hand, it might prolong the agonal phase by preventing collapse of upper airway of the potential donor (7).

In a pig DCD model Sanchez *et al.* showed that pre-arrest heparin administration improved organ function by preserving endothelial homeostasis (61). Contrary to this report, Keshava *et al.* demonstrated that DCD lungs could be used regardless of ante-mortem heparin administration (62). To date there is no clinical study to compare pre-mortem heparin use versus no heparin use. There are some centers that use pre-mortem heparin in a potential DCD donor (17,20,23,24,28,60,63). However some centers do not use premortem heparin (19,21,26,59).

Agonal phase is defined as the time period between withdrawal of life support and cardiac arrest. Although there is not a consensus about the optimal time period among the centers, this period varies from 30 to 180 minutes (17,19-21,23,25,26,59,60,63,64). Most of the centers are allowing

maximum time of 90 minutes.

Tolerable WIT, defined as the time between cardiac arrest and cold flush, is around 30 minutes (5,7,10-12,17,19-26,28,59,60,63,64). However, based on experimental data WIT of 60 minutes is tolerable (1,7).

EVLP

The EVLP is as a technology to evaluate and recondition lung graft before transplantation (10,16,58,65). Originally, EVLP has been proposed to assess the function of the lung from an uncontrolled DCD donor (category II) as an interim evaluation of the graft prior to transplantation (58). The Toronto Group modified this method and published their results in nine cDCD donors (66). Selective use of EVLP is a part of the DCD Program in most centers (20,59,66).

The exact role of EVLP in category III DCD has not been established (67). Excellent results have been obtained without the routine use of EVLP (17). In contrast, EVLP may help to exclude lungs with injuries that have not been recognized after withdrawal of life support therapies and may help for acceptance of longer agonal times (67).

uDCD donors

Steen *et al.* in Sweden performed the first successful LTx from an uDCD after evaluation with EVLP (58). The Madrid Group is the center with the largest experience on uDCD donation (14,27). Standard criteria for uDCD donation used by Madrid Group are shown in *Table 5* (14,27).

Madrid Group recently reported 29 lung transplants from uDCD donors (category II) (14). Overall hospital mortality rate was 17%. Survival rates at 1, 2 and 5 years were 68%, 57% and 51%, respectively. The cumulative incidence of bronchiolitis obliterans syndrome (BOS) was 11%, 35% and 45% at 1, 3 and 5 years, respectively (14). Use of EVLP in uDCD donors is strongly recommended (68).

Selective EVLP use in uDCD donors is suggested from Spanish Group according to the following situations (14):
(I)   PaO$_2$/FiO$_2$ <400 mmHg;
(II)   Signs of pulmonary edema on chest X-ray or during procurement;
(III) Poor lung compliance at the procurement;
(IV)   Donors: >65 years old, questionable history of aspiration, heavy smoker, expected long ischemic time.

Table 5 Standard criteria for uDCD donation used by Madrid Group (14,27)

Age <65 years

Smoking <20 pack/years

Appropriate size matching with the recipient

  Blood group compatibility

  Absence of cardiopulmonary surgery

  Absence of aspiration on bronchoscopy

  Chest X-ray: absence of pulmonary edema, infection

  Adequate blood gas measurement with single flush technique (PaO_2/FiO_2 >400 mmHg)

  Topical cooling (target pleural temperature <21 ℃)

Time sequence

  No touch period after cardiac arrest ≤15 min

  Warm ischemic time (cardiac arrest—topical cooling) ≤100 min

  Total time of topical cooling ≤240 min

Outcomes from cDCD donor LTx

Levvey et al. reported 5-year results of 72 category III DCD LTx reported to the Australian National DCD Lung Transplant Collaborative (17). One- and 5-year actuarial survival was 97% and 90% in DCD, vs. 90% and 61%, for 503 DBD lung transplants, respectively (17).

Recently, Leuven Group updated their DCD LTx series in 59 recipients (56). The comparison was done with a cohort of DBD LTx recipients (n=331). There was no difference in time on mechanical ventilation, ICU stay, highest PGD score and hospital stay. Moreover, chronic lung allograft dysfunction (CLAD)-free and overall survival did not differ between the DBD and DCD group (56).

Erasmus et al. from Groningen evaluated the effectiveness of DCD LTx from 35 category III DCD donors (19). Five-year survival was 73% in DCD and 66% in DBD cohorts. Survival, occurrence of PGD, and acute rejection was comparable to the DBD cohort. The incidence of BOS was lower in the DCD group (19).

Mason et al. using data from the United Network for Organ Sharing (UNOS) for LTx compared (I) survival after LTx of recipients of DCD versus DBD donor organs in the United States and (II) recipient characteristics (24). Among 14,939 transplants that were performed, 36 were DCD. Unadjusted survival at 1, 6, 12, and 24 months was 94%, 94%, 94%, and 87%, respectively, for DCD donors versus 92%, 84%, 78%, and 69%, respectively, for DBD donors (P=0.04).

De Oliveira et al. from University of Wisconsin showed that the long-term patient and graft survival rates after DCD LTx were equivalent to those after DBD LTx (60).

St. Louis Group also reported that at their center, early outcomes after DCD LTx were reported to be somewhat inferior to those of series from other centers but approach national averages for conventional LTx (21).

Data from the ISHLT DCD Registry was recently published (12). There were 306 transplants performed using DCD donors and 3,992 transplants using DBD donors during the study period. Median age for DCD donors was 44 years (range, 16–62 years) and 40 years (range, 15–64 years) for DBD donors. Heparin was given in 54% of the cases, donor extubation occurred in 90% of the cases, and selective normothermic EVLP was used in 12%. The median time from withdrawal of life support therapy (WLST) to cardiac arrest was 15 minutes (5th to 95th percentiles of 5 to 55 minutes), and from WLST to cold flush was 33 minutes (5th to 95th percentiles of 19.5 to 79.5 minutes). Thirty-day survival was 96% in the DCD group and 97% in the DBD group. One-year survival was 89% in the DCD group and 88% in the DBD group. Five-year survival was 61% in both groups (12). In order to standardize the definitions around important times in DCD donation process, ISHLT DCD Working Group recommended the following times points and intervals (12) (Table 3, Figure 1). No differences in 1-year survival were observed for the different lengths of intervals 1 and 2 (<10 vs. 10 to 20 vs. 420 minutes; P=0.36 and P=0.83 for intervals 1 and 2, respectively). Similarly, no differences in survival were observed for interval 3 duration (<30 vs. 30 to 45 vs. 445 minutes; P=0.11). There was no significant correlation between the interval of WLST to pulmonary flush with survival (P=0.11) (12).

Recently, Sabashnikov et al. from Harefield investigated long-term outcomes after LTx with DCD donors in comparison with those obtained from DBD donors (64). There were no significant differences regarding intraoperative variables and total ischemic time. Patients from the DCD group had significantly higher incidence of primary graft dysfunction grade 3 at the end of the procedure (P=0.014), and significantly lower PaO_2/FiO_2 ratio during the first 24 h after the procedure (P=0.018). There was a trend towards higher incidence of the need for postoperative extracorporeal life support in the DCD group. While the overall cumulative survival was not significantly different,

Table 6 Zurich University Hospital DCD category III LTx program characteristics

Age <70 years
Chest X-ray: no infiltrations (if necessary perform thorax CT)
Adequate blood gas measurement (PaO_2/FiO_2 >400 mmHg)
Pre-mortem heparin: yes
Pre-mortem bronchoscopy: yes
Placement of nasogastric tube: yes
Stand-off period: 10 min
Length of agonal phase (WLST to cardiac arrest): 60 min (will be increased to 120 min)
Withdrawal of tracheal tube: yes
Before extubation aspiration of nasogastric tube
After stand-off period; intubation and ventilation
Maximal length of warm ischemic time (cardiac arrest to cold flush): 60 min
Selective use of EVLP: yes
No signs of aspiration during retrieval bronchoscopy
Extended criteria donors such as smoking history of >20 pack/years, ICU stay >5 days, PaO_2/FiO_2 <400 mmHg and abnormal chest X-ray are also accepted

the DCD group had significantly poorer results in terms of BOS-free survival in the long-term follow-up (64). They concluded that long-term results after LTx from DCD are in general comparable with those obtained after DBD LTx. However, patients transplanted using organs from DCD donors have a predisposition for development of BOS in the longer follow-up (64).

DCD category III LTx program in Switzerland

Following the legal regulations, utilization of DCD (category III) donors is allowed in Switzerland (1st September 2011). SwissTransplant Working Group on DCD organized multiple meetings. Zurich University Hospital constituted a working group for multiorgan DCD Program. According to our local committee (DCD Working Group) in Zurich, we decided to perform first three DCD category III donors only for kidneys, 4th and 5th for liver, followed by lung retrieval. We performed the first lung DCD LTx in February 2012. As of April 2017, we performed 21 LTxs from DCD donors. Zurich DCD

LTx Program details are given in *Table 6*. We presented the results of the first 19 cases at ISHLT 37th Annual Meeting and Scientific Sessions in San Diego, USA, in April 2017 (69).

In our series, median agonal phase (withdrawal-cardiac arrest) was 17 minutes [interquartile range (IQR), 11–20 minutes]. Median donor oxygenation capacity was 48 kPa (IQR, 40–52 kPa). Median WIT (cardiac arrest-cold perfusion) was 31 minutes (IQR, 24–37 minutes). Intraoperative extracorporeal membrane oxygenation (ECMO) was used in seven recipients, two of them were bridged to transplantation on ECMO. In two DCDs normothermic *ex vivo* lung perfusion was done before implantation. The median intubation time was 1 day (IQR, 1–2 days). ICU time was 3 days (IQR, 2–5 days). Two patients developed primary graft dysfunction grade 3 within 72 h. The 90-day mortality in DCD group was 0%. Actuarial survival rates at 1 and 3 years are 100% and 79% for DCD and 85% and 67% for the DBD group, respectively (P=0.5).

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Van Raemdonck DE, Rega FR, Neyrinck AP, et al. Non-heart-beating donors. Semin Thorac Cardiovasc Surg 2004;16:309-21.
2. Egan TM. Non-heart-beating donors in thoracic transplantation. J Heart Lung Transplant 2004;23:3-10.
3. Thuong M, Ruiz A, Evrard P, et al. New classification of donation after circulatory death donors definitions and terminology. Transpl Int 2016;29:749-59.
4. Reeb J, Keshavjee S, Cypel M. Successful lung transplantation from a donation after cardiocirculatory death donor taking more than 120 minutes to cardiac arrest after withdrawal of life support therapies. J Heart Lung Transplant 2016;35:258-9.
5. Mooney JJ, Hedlin H, Mohabir PK, et al. Lung Quality and Utilization in Controlled Donation After Circulatory Determination of Death Within the United States. Am J Transplant 2016;16:1207-15.

6. Iyer A, Chew HC, Gao L, et al. Pathophysiological Trends During Withdrawal of Life Support: Implications for Organ Donation After Circulatory Death. Transplantation 2016;100:2621-9.

7. Erasmus ME, van Raemdonck D, Akhtar MZ, et al. DCD lung donation: donor criteria, procedural criteria, pulmonary graft function validation, and preservation. Transpl Int 2016;29:790-7.

8. Dark JH, Egan TM. Lungs From the Controlled Donation After Circulatory Determination of Death Donor: Perspectives From the United States and Beyond. Am J Transplant 2016;16:1047-8.

9. Dark JH. Lung transplantation from donation after cardiocirculatory death: the end of the golden era? Eur J Cardiothorac Surg 2016;49:53-4.

10. Reeb J, Keshavjee S, Cypel M. Expanding the lung donor pool: advancements and emerging pathways. Curr Opin Organ Transplant 2015;20:498-505.

11. Krutsinger D, Reed RM, Blevins A, et al. Lung transplantation from donation after cardiocirculatory death: a systematic review and meta-analysis. J Heart Lung Transplant 2015;34:675-84.

12. Cypel M, Levvey B, Van Raemdonck D, et al. International Society for Heart and Lung Transplantation Donation After Circulatory Death Registry Report. J Heart Lung Transplant 2015;34:1278-82.

13. Cypel M, Keshavjee S. Strategies for safe donor expansion: donor management, donations after cardiac death, ex-vivo lung perfusion. Curr Opin Organ Transplant 2013;18:513-7.

14. Gomez-de-Antonio D, Campo-Canaveral JL, Crowley S, et al. Clinical lung transplantation from uncontrolled non-heart-beating donors revisited. J Heart Lung Transplant 2012;31:349-53.

15. Wigfield CH, Love RB. Donation after cardiac death lung transplantation outcomes. Curr Opin Organ Transplant 2011;16:462-8.

16. Cypel M, Yeung JC, Keshavjee S. Novel approaches to expanding the lung donor pool: donation after cardiac death and ex vivo conditioning. Clin Chest Med 2011;32:233-44.

17. Levvey BJ, Harkess M, Hopkins P, et al. Excellent clinical outcomes from a national donation-after-determination-of-cardiac-death lung transplant collaborative. Am J Transplant 2012;12:2406-13.

18. Aigner C, Slama A, Hotzenecker K, et al. Clinical ex vivo lung perfusion--pushing the limits. Am J Transplant 2012;12:1839-47.

19. Van De Wauwer C, Verschuuren EA, van der Bij W, et al. The use of non-heart-beating lung donors category III can increase the donor pool. Eur J Cardiothorac Surg 2011;39:e175-80; discussion e80.

20. De Vleeschauwer SI, Wauters S, Dupont LJ, et al. Medium-term outcome after lung transplantation is comparable between brain-dead and cardiac-dead donors. J Heart Lung Transplant 2011;30:975-81.

21. Puri V, Scavuzzo M, Guthrie T, et al. Lung transplantation and donation after cardiac death: a single center experience. Ann Thorac Surg 2009;88:1609-14; discussion 14-5.

22. De Vleeschauwer S, Van Raemdonck D, Vanaudenaerde B, et al. Early outcome after lung transplantation from non-heart-beating donors is comparable to heart-beating donors. J Heart Lung Transplant 2009;28:380-7.

23. Cypel M, Sato M, Yildirim E, et al. Initial experience with lung donation after cardiocirculatory death in Canada. J Heart Lung Transplant 2009;28:753-8.

24. Mason DP, Thuita L, Alster JM, et al. Should lung transplantation be performed using donation after cardiac death? The United States experience. J Thorac Cardiovasc Surg 2008;136:1061-6.

25. Mason DP, Murthy SC, Gonzalez-Stawinski GV, et al. Early experience with lung transplantation using donors after cardiac death. J Heart Lung Transplant 2008;27:561-3.

26. Zych B, Popov AF, Amrani M, et al. Lungs from donation after circulatory death donors: an alternative source to brain-dead donors? Midterm results at a single institution. Eur J Cardiothorac Surg 2012;42:542-9.

27. de Antonio DG, Marcos R, Laporta R, et al. Results of clinical lung transplant from uncontrolled non-heart-beating donors. J Heart Lung Transplant 2007;26:529-34.

28. Hernandez-Alejandro R, Wall W, Jevnikar A, et al. Organ donation after cardiac death: donor and recipient outcomes after the first three years of the Ontario experience. Can J Anaesth 2011;58:599-605.

29. Hardy JD, Webb WR, Dalton ML Jr, et al. Lung Homotransplantation in Man. JAMA 1963;186:1065-74.

30. Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary fibrosis. N Engl J Med 1986;314:1140-5.

31. Egan TM, Lambert CJ Jr, Reddick R, et al. A strategy to increase the donor pool: use of cadaver lungs for transplantation. Ann Thorac Surg 1991;52:1113-20; discussion 20-1.

32. D'Armini AM, Roberts CS, Griffith PK, et al. When does

the lung die? I. Histochemical evidence of pulmonary viability after "death". J Heart Lung Transplant 1994;13:741-7.

33. D'Armini AM, Tom EJ, Roberts CS, et al. When does the lung die? Time course of high energy phosphate depletion and relationship to lung viability after "death". J Surg Res 1995;59:468-74.

34. Ulicny KS Jr, Egan TM, Lambert CJ Jr, et al. Cadaver lung donors: effect of preharvest ventilation on graft function. Ann Thorac Surg 1993;55:1185-91.

35. Egan TM, Ulicny KS Jr, Lambert CJ Jr, et al. Effect of a free radical scavenger on cadaver lung transplantation. Ann Thorac Surg 1993;55:1453-9.

36. Roberts CS, Hennington MH, D'Armini AM, et al. Donor lungs from ventilated cadavers: impart of a free radical scavenger. J Heart Lung Transplant 1996;15:275-82.

37. Hennington MH, D'Armini AM, Lemasters JJ, et al. Cadaver lungs for transplantation. Effect of ventilation with alveolar gas. Transplantation 1996;61:1009-14.

38. Rega FR, Wuyts WA, Vanaudenaerde BM, et al. Nebulized N-acetyl cysteine protects the pulmonary graft inside the non-heart-beating donor. J Heart Lung Transplant 2005;24:1369-77.

39. Rega FR, Jannis NC, Verleden GM, et al. Should we ventilate or cool the pulmonary graft inside the non-heart-beating donor? J Heart Lung Transplant 2003;22:1226-33.

40. Rega FR, Vanaudenaerde BM, Wuyts WA, et al. IL-1beta in bronchial lavage fluid is a non-invasive marker that predicts the viability of the pulmonary graft from the non-heart-beating donor. J Heart Lung Transplant 2005;24:20-8.

41. Dougherty JC, Sinha S, Kibble F, et al. Intolerance of the ischemic lung to hypothermic ventilation. J Appl Physiol 1972;32:632-4.

42. Watanabe S, Sakasegawa K, Shimokawa S, et al. Intrathoracic cooling of cadavers before lung transplantation using cold air: an experimental study. Transplantation 2002;73:39-43.

43. Steen S, Ingemansson R, Budrikis A, et al. Successful transplantation of lungs topically cooled in the non-heart-beating donor for 6 hours. Ann Thorac Surg 1997;63:345-51.

44. Steen S, Liao Q, Wierup PN, et al. Transplantation of lungs from non-heart-beating donors after functional assessment ex vivo. Ann Thorac Surg 2003;76:244-52; discussion 52.

45. Yoshida S, Sekine Y, Shinozuka N, et al. The efficacy of partial liquid ventilation in lung protection during hypotension and cardiac arrest: preliminary study of lung

46. Okazaki M, Date H, Inokawa H, et al. Optimal time for post-mortem heparinization in canine lung transplantation with non-heart-beating donors. J Heart Lung Transplant 2006;25:454-60.

47. Inci I, Zhai W, Arni S, et al. Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors. J Heart Lung Transplant 2007;26:1054-60.

48. Inci I, Arni S, Inci D, et al. Impact of topical cooling solution and prediction of pulmonary graft viability from non-heart-beating donors. J Heart Lung Transplant 2008;27:1016-22.

49. Inci I, Arni S, Acevedo C, et al. Surfactant alterations following donation after cardiac death donor lungs. Transpl Int 2011;24:78-84.

50. Inci I, Hillinger S, Arni S, et al. Reconditioning of an injured lung graft with intrabronchial surfactant instillation in an ex vivo lung perfusion system followed by transplantation. J Surg Res 2013;184:1143-9.

51. Martens A, Boada M, Vanaudenaerde BM, et al. Steroids can reduce warm ischemic reperfusion injury in a porcine donation after circulatory death model with ex vivo lung perfusion evaluation. Transpl Int 2016;29:1237-46.

52. Huerter ME, Sharma AK, Zhao Y, et al. Attenuation of Pulmonary Ischemia-Reperfusion Injury by Adenosine A2B Receptor Antagonism. Ann Thorac Surg 2016;102:385-93.

53. Kootstra G, Daemen JH, Oomen AP. Categories of non-heart-beating donors. Transplant Proc 1995;27:2893-4.

54. Levvey BJ, Westall GP, Kotsimbos T, et al. Definitions of warm ischemic time when using controlled donation after cardiac death lung donors. Transplantation 2008;86:1702-6.

55. Snell GI, Oto T, Levvey B, et al. Evaluation of techniques for lung transplantation following donation after cardiac death. Ann Thorac Surg 2006;81:2014-9.

56. Ruttens D, Martens A, Ordies S, et al. Short- and Long-Term Outcome After Lung Transplantation From Circulatory-Dead Donors: A Single-Center Experience. Transplantation 2017. [Epub ahead of print].

57. Snell GI, Levvey BJ, Oto T, et al. Early lung transplantation success utilizing controlled donation after cardiac death donors. Am J Transplant 2008;8:1282-9.

58. Steen S, Sjoberg T, Pierre L, et al. Transplantation of lungs from a non-heart-beating donor. Lancet 2001;357:825-9.

59. Erasmus ME, Verschuuren EA, Nijkamp DM, et al. Lung transplantation from nonheparinized category

III non-heart-beating donors. A single-centre report. Transplantation 2010;89:452-7.

60. De Oliveira NC, Osaki S, Maloney JD, et al. Lung transplantation with donation after cardiac death donors: long-term follow-up in a single center. J Thorac Cardiovasc Surg 2010;139:1306-15.

61. Sanchez PG, Bittle GJ, Williams K, et al. Ex vivo lung evaluation of prearrest heparinization in donation after cardiac death. Ann Surg 2013;257:534-41.

62. Keshava HB, Farver CF, Brown CR, et al. Timing of heparin and thrombus formation in donor lungs after cardiac death. Thorac Cardiovasc Surg 2013;61:246-50.

63. Mason DP, Brown CR, Murthy SC, et al. Growing single-center experience with lung transplantation using donation after cardiac death. Ann Thorac Surg 2012;94:406-11; discussion 11-2.

64. Sabashnikov A, Patil NP, Popov AF, et al. Long-term results after lung transplantation using organs from circulatory death donors: a propensity score-matched analysisdagger. Eur J Cardiothorac Surg 2016;49:46-53.

65. Yeung JC, Cypel M, Waddell TK, et al. Update on donor assessment, resuscitation, and acceptance criteria, including novel techniques--non-heart-beating donor lung retrieval and ex vivo donor lung perfusion. Thorac Surg Clin 2009;19:261-74.

66. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. N Engl J Med 2011;364:1431-40.

67. Cypel M, Levvey B, Van Raemdonck D, et al. Lung transplantation using controlled donation after circulatory death donors: Trials and tribulations. J Heart Lung Transplant 2016;35:146-7.

68. Egan TM, Requard JJ 3rd. Uncontrolled Donation After Circulatory Determination of Death Donors (uDCDDs) as a Source of Lungs for Transplant. Am J Transplant 2015;15:2031-6.

69. Inci I, Lenherr R, Hillinger S, et al. Lung Transplantation with Controlled Donation After Circulatory Death Donors: Initial Experience in Switzerland. J Heart Lung Transplant 2017;36:S319.

Pathophysiology and classification of primary graft dysfunction after lung transplantation

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Abstract: The term primary graft dysfunction (PGD) incorporates a continuum of disease severity from moderate to severe acute lung injury (ALI) within 72 h of lung transplantation. It represents the most significant obstacle to achieving good early post-transplant outcomes, but is also associated with increased incidence of bronchiolitis obliterans syndrome (BOS) subsequently. PGD is characterised histologically by diffuse alveolar damage, but is graded on clinical grounds with a combination of PaO2/FiO2 (P/F) and the presence of radiographic infiltrates, with 0 being absence of disease and 3 being severe PGD. The aetiology is multifactorial but commonly results from severe ischaemia-reperfusion injury (IRI), with tissue-resident macrophages largely responsible for stimulating a secondary 'wave' of neutrophils and lymphocytes that produce severe and widespread tissue damage. Donor history, recipient health and operative factors may all potentially contribute to the likelihood of PGD development. Work that aims to minimise the incidence of PGD in ongoing, with techniques such as *ex vivo* perfusion of donor lungs showing promise both in research and in clinical studies. This review will summarise the current clinical status of PGD before going on to discuss its pathophysiology, current therapies available and future directions for clinical management of PGD.

Keywords: Primary graft dysfunction (PGD); oedema; ischaemia-reperfusion injury (IRI); inflammation; ex vivo lung perfusion (EVLP)

Definition of primary graft dysfunction (PGD)

PGD is a syndrome that encompasses a spectrum of mild to severe lung injury that occurs within the first 72 h after lung transplantation. PGD is a major cause of early morbidity and mortality in lung transplantation and is characterised by progressive hypoxaemia and alveolar infiltrates on a chest radiograph. Following ischaemia in the donor organ and reperfusion in the recipient, inflammatory and immunological injury-repair responses seem to be the key pathological mechanisms. PGD has significant impact on the short and longer-term outcomes for lung transplant patients, however strategies aimed at identifying and reducing PGD risk are being developed. Ex vivo lung perfusion (EVLP) is one strategy to improve assessment of the donor organ and has the potential to act as a platform for implementing interventions to reduce the risk of or prevent PGD. This review will provide an overview of the pathophysiology of PGD and its implications in the clinical setting.

In 2005, the International Society for Heart and Lung Transplantation (ISHLT) published their standardised definition for PGD (updated in 2016) (1). In this definition, the PaO_2/FiO_2 (P/F) ratio and the presence of bilateral

Table 1 ISHLT PGD definition and severity grading 2005 and updates from 2016

PGD stage	P/F ratio (mmHg)	Chest radiography	Updates from 2016 Consensus Group
0	>300	Normal	Any P/F ratio
1	>300	Diffuse allograft infiltration/pulmonary oedema	No changes
2	200–300	Diffuse allograft infiltration/pulmonary oedema	No changes
3	<200	Diffuse allograft infiltration/pulmonary oedema	No changes

ISHLT, the International Society for Heart and Lung Transplantation; PGD, primary graft dysfunction; P/F, PaO_2/FiO_2.

infiltrates on a chest radiograph consistent with non-cardiogenic pulmonary oedema are assessed, see *Table 1*. Assessment is carried out at specific time points after reperfusion; within the first 6 h (T0), post 24 h (T24), 48 h (T48) and 72 h (T72). Ideally, the P/F ratio is measured on a FiO_2 of 1.0 and positive end expiratory pressure (PEEP) of 5 cm $H20$. The radiographic findings of PGD are non-specific and include peri-hilar ground glass opacities, peri-bronchial, perivascular thickening and reticular interstitial and airspace opacities located in a dependent fashion. The 2016 Consensus Group has clarified that the PGD timing starts at the point of reperfusion following release of the second lung recipient pulmonary arterial cross clamp. The Consensus Group proposes that no changes be made to the grading time points of T0, T24, T48, and T72 and reaffirms this approach and now clarifies that "any" P/F ratio is to be considered grade 0 in the absence of radiographic diffuse pulmonary oedema.

Other contributory factors that can mimic, modify and confound the definition and grading need to be excluded, including cardiogenic oedema, pneumonia, hyper-acute rejection and pulmonary venous anastomotic obstruction.

Epidemiology and clinical outcomes

Historically it was difficult to accurately assess the incidence of PGD due to varying definitions and even with the introduction of the ISHLT standardised definition in 2005; there is still a dependence on accuracy in the timing and severity grading of PGD. Since the implantation of the standardised ISHLT definition, the incidence of severe PGD grade 3 (PGD3) within 48–72 h postoperatively has been reported at approximately 10–20% and the incidence of PGD3 at any time point within the first 3 days, approximately 30% (2-4). Diamond *et al.* demonstrated in a large, multi-centre, prospective cohort study of 1,255 lung transplant recipients between 2002 and 2010, that there was

an overall incidence of PGD grade 3 of 30.8% at any time point during the first 72 h of lung transplantation, while grade 3 PGD present at 48 or 72 h after reperfusion had an incidence of 16.8% (5).

PGD is associated with significant early and late post-transplant morbidity: patients with PGD3 have longer hospital and intensive care length of stays, duration of mechanical ventilation, increased short and longer-term mortality than those with lower grades of PGD. Whitson *et al.* analysed 402 lung transplant recipients at the University of Minnesota from 1992 to 2004, reporting that that the 90-day mortality rate associated with the occurrence of PGD grade 3 at any point within the first 48 h after transplantation was 17% versus 9% in the group without grade 3 PGD (6). In a study of the United Network for Organ Sharing (UNOS)/ISHLT database between 1994 and 2000, all-cause mortality at 1-year was 64.9% in recipients with PGD 3 beyond 48 h versus 20.4% in the non-PGD group (7). Diamond *et al.* showed that PGD was significantly associated with 90-day and 1-year mortality with grade 3 PGD at 48 or 72 h after transplant being associated with an absolute risk increase for death within 90 days compared to those without grade 3 PGD of 18% and 23 % for death within 1 year (5). PGD has also been associated with an increased risk of developing bronchiolitis obliterans syndrome (BOS) which is the main limiting factor for long-term survival after lung transplantation (8).

Whitson *et al.* showed that patients with PGD3 had a significantly reduced longer term survival compared with those PGD1 and PGD2; median survival grade 3: 4.6 years; grade 2: 6.6 years; grade 1: 7.5 years (6). Kreisel *et al.* reported significant association between PGD with decreased long-term survival in their single centre cohort study of 1,000 recipients. They showed 1-, 5- and 10-year survival to be 72.8%, 43.9 % and 18.7% in the PGD group compared with 87.1%, 59.8% and 35.7% in the non-PGD group respectively (9). PGD has also been shown to

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significantly impact on recipient functional status which ultimately will impact on quality of life. For example, survivors of PGD 3 at 12 months were seen to have a much shorter 6 min walk distance than those without (10).

Risk factors: pre-transplantation

With regard to the overall lung transplant procedure there are several places that can act as potential risk factors for subsequent PGD. Brain death leads to haemodynamic compromise, hormonal derangements, hypothermia and release of inflammatory cytokines (11,12). Warm ischaemia occurring after loss of circulation during organ retrieval and then cold ischaemia to preserve the organs causes a deterioration in tissue oxygenation. A release of inflammatory cytokines, namely IL-8, IL-12, IL-18, TNF-alpha and IFN-gamma triggers a cascade of tissue apoptosis, necrosis and results in organ dysfunction (11). The pathophysiology will be discussed in depth later in this review.

Hypoxia during ischaemic periods promotes coagulation through activation of endothelial cells, leading to a pro-coagulable environment. In an analysis of the UNOS database, an association with PGD was found with donor traumatic brain injury (TBI) as a cause of death (13). Diamond *et al.*, however reported in a large multicentre cohort study that cause of donor death was not a risk factor for PGD (5).

There are increasing numbers of lung transplantations following donation after circulatory confirmation of death (DCD), with differing opinions on the effect of type of donation, DCD versus to donation after brain death (DBD) on PGD risk. A meta-analysis of five studies found no difference in PGD incidence between DCD and DBD donors (14). A study published the same year, however conversely reported that there was a higher incidence of PGD and a trend towards greater need for extracorporeal life support (ECMO) in a series of 60 DCD transplant recipients compared to their score matched DBD recipients (15).

Risk factors: post-transplantation

Donor-related factors

Donor-related factors can be categorised as hereditary and acquired. PGD risk has been reported to increase in donors older than 32–45 years (16). Whitson *et al.* demonstrated that the relationship between the development of PGD3

and donor age in their series was linear from the age 35 years upwards and a 3% increased risk of PGD per one-year increased in donor age (6). More recent data from Baldwin *et al.* suggests that the age-related risk of PGD is restricted to the extremes of age and that donor age 55 to 64 years was not associated with a significantly increased risk of severe PGD after controlling for recipient, surgical and other donor factors (17).

Other donor risk factors reported include age, African American race, female gender, and history of tobacco exposure (6,12,13,16,18). Bonser *et al.* reviewed the effect of donor smoking after lung transplantation using a cohort study of a prospective registry and found that donor smoke exposure was associated with worse recipient outcomes (19). The survival probability, however, still exceeded that of remaining on the waiting list. Alcohol use also appears to increase PGD risk, with donors categorised as "heavy drinkers" displaying a 9-fold higher risk of severe PGD compared to those with no alcohol intake (20).

Potential donor risk-factors that are acquired include prolonged periods of mechanical ventilation, aspiration, pneumonia, excessive blood transfusion and trauma, however further definitive studies are needed to demonstrate their associations with PGD. The presence of fat embolism from the donor is said to increases the risk of PGD development by 25-fold (21).

Recipient-related factors

The primary diagnosis leading to the need for lung transplantation is an important modifier of the risk of developing PGD. The most significant recipient risk factors are obesity (22,23), pulmonary hypertension and diagnoses of pulmonary fibrosis (24) and/or sarcoidosis (6,25). In a systematic review of 10 studies, the incidence of PGD was 11.8% in patients with chronic obstructive pulmonary disease (COPD), 12.4% in cystic fibrosis, 18.0% in patients with idiopathic pulmonary fibrosis (IPF), 50% in sarcoidosis and 30.3% in patients with idiopathic pulmonary arterial hypertension (IPAH) (18). Several studies including a meta-analysis, large multicentre cohort study and a single centre study have shown IPAH was strongly associated with risk for PGD. This remains the case even after adjustment for recipient pulmonary artery systolic pressure, with reports highlighting that higher pulmonary arterial pressure has been shown to be strongly correlated with the development of PGD, with a 30% increased risk of PGD for every 10 mmHg increase in mean PAP (5,16,18). After adjustment

for multiple risk factors, obese and overweight recipient body mass index (BMI) were independent predictors of PGD. Lederer *et al.* demonstrated a link between plasma levels of leptin which is associated with adiposity and risk of PGD with obesity being associated with greater than a 2-fold increased risk of grade 3 PGD within 72 h postoperatively in adult lung transplant recipients with COPD or ILD (22).

Pre-transplantation biological levels of certain markers have also been shown to have an association with a higher risk of PGD development. Cantu and colleagues found that donor polymorphisms in the oxidant stress gene *NOX3* were associated with increased risk of PGD (26). Machuca and colleagues found that lungs that subsequently developed PGD had higher levels of IL-8, macrophage colony stimulating factor and growth-related oncogene-α compared to lungs that did not develop PGD (27). More PGD-associated biomarkers will be discussed later on in the review.

Operative/surgical factors

The operative risk factors for PGD reported are single lung transplant procedure, prolonged ischaemia time, cardiopulmonary bypass (CPB) use, red blood cell transfusion greater than 1 litre and reperfusion FiO_2 greater than 0.4 (5,13,28-30). Weber *et al.* reported that patients who received an intraoperative blood transfusion of greater than 4 units were seen to have an increase in PGD, renal replacement therapy, a post-operative ECMO requirement and mortality (31). CPB has been associated with an increased risk of PGD, reported by Aeba *et al.* in a retrospective study over a 2-year period in 100 lung transplant recipients. They found that the cohort of patients whom had CPB had a more prolonged period of intubation and more severe pulmonary infiltrates on radiography (32). Diamond *et al.* demonstrated in their large, multi-centre cohort study and meta-analysis that CPB was an independent risk factor for the development of PGD (5) and also reported the association between PGD and single lung transplantation. In a meta-analysis, however this did not seem to be the case (18).

Reperfusion FiO_2 was also seen to be an independent risk factor for PGD in a large, 10-centre prospective cohort study with an increased risk of PGD at 48 or 72 h reported in patients where the reperfusion FiO_2 had been >0.4, with an absolute risk increase of 6% when compared to a reperfusion FiO_2 of <0.4 (5).

Pathophysiology of primary graft dysfunction

The underlying causes of PGD remain poorly defined. However, the emergence of data from large, multicentre-derived lung transplant recipient outcome studies and genetic data sets has allowed for the development in the understanding of the PGD mechanisms at a cellular and molecular level.

Neutrophil activation and interactions with the endothelium

The hallmark of PGD pathophysiology is the migration of polymorphonuclear neutrophils (PMNs) from the pulmonary circulation into interstitium and the airways. These PMNs are attracted out of the circulation by chemotactic mediators such as CXCL8 and damage-associated molecular patterns (DAMPs) which have been released from apoptotic and necrotic lung tissue following ischaemia-reperfusion injury (IRI). This leads to the release of DAMPs into the circulation, with HMGB1 and ATP being well-known examples. A hyperoxic mouse model of acute lung injury (ALI) utilised by Entezari *et al.* identified high levels of HMGB1 in the bronchoalveolar lavage fluid (BALF) of these mice (33). Intra-tracheal administration of additional HMGB1 within this model served to further exacerbate the symptoms of ALI in this model by mediating leukocyte infiltration into the airways. Shah *et al.* used another model of pulmonary inflammation to identify extracellular ATP as another potent mediator of neutrophil recruitment into the lungs during ALI (34). Both HMGB1 and ATP are well-renowned DAMPs, thus IRI-mediated damage of the pulmonary airways serves to release these into the circulation and facilitate attraction of neutrophils to the lungs in the case of acute pulmonary diseases such as PGD.

Alongside neutrophil activation and migration, activation of pulmonary endothelium is another very important part of the pathophysiology of PGD. This activation occurs through the release of pro-inflammatory cytokines TNFα and IL-1β which enables up-regulation of adhesion molecules on the surface of the endothelium (35,36). E-selectin on the endothelial surface interacts with P- and L-selectin found on neutrophils to facilitate weak binding, as the latter roll along the endothelial surface through a cytokine 'gradient' before being arrested via integrin binding. Mac-1 and $\alpha_4\beta_1$ have been shown to play essential roles in recruiting neutrophils to the lungs during *S. Pneumoniae* infection and thus likely enable this process

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in the case of inflammation produced in PGD (37). Once localised to the lungs, transmigration of PMNs into the interstitial space and airways is facilitated by up-regulation of adhesion molecules, notably ICAM-1 and PECAM-1 (36). Support for the significance of the models is demonstrated by the study conducted by Simms *et al.*, who identified heightened CD11b/CD18 cell surface expression as being associated with individuals who displayed acute respiratory distress syndrome (ARDS) (38). Finally, a study that utilised atopic asthma patients highlighted the fact that a CXCR1/2 agonist was able to down-regulate the amount of CXCL8-mediated neutrophil infiltration of the airways, indicating that this is a key pathway by which neutrophil trafficking in the context of inflammatory lung disease is enabled (39). Thus, the combination of *in vivo* models of neutrophil trafficking along with epidemiological studies of associated molecular entities highlight the enormous significance of this process in enabling heightened pulmonary inflammation.

Endothelial cells up-regulate adhesion markers and secrete cytokines in response to IRI, resulting in leukocyte recruitment out of the circulation. The act of interrupting blood flow before restoration is considered the major event in IRI that disrupts the homeostasis of the endothelium. This is a well-documented mechanism in which the sudden re-establishment of blood flow within the organ causes dramatic cell depolarization. A 2011 study of 126 PGD patients between the year 2002 and 2007 was carried out by Fang *et al.* that investigated the effect of pulmonary arterial pressure on the likelihood of developing grade 3 PGD. The authors found a statistically significant correlation between the two, indicating the effect of significant reperfusion force on endothelial integrity (24). Schnickel *et al.* analyzed the effects of a modified reperfusion technique involving the insertion of a catheter into the main or individual pulmonary artery after implantation on the incidence of PGD (40). The recipient blood was depleted of leukocytes; supplemented with nitroglycerin; adjusted for pH and calcium level; enriched with aspartate, glutamate, and dextrose; then administered into the pulmonary arteries of the newly transplanted lung(s) for the first 10 min of reperfusion. Severe primary graft dysfunction was defined as a PaO_2/inspired oxygen fraction of less than 150 with diffuse infiltrate on the radiograph in absence of other causes. One hundred patients underwent lung transplantation with the modified reperfusion technique. Forty-two patients underwent single-lung transplantation, of which 5 patients required CPB for the procedure. Fifty-eight patients

underwent double-lung transplantation; all double-lung transplantation procedures were performed with patients on CPB. There were no technical complications associated with the modified reperfusion, with the mean PaO_2/inspired oxygen fraction at 6 h in this cohort being 252±123 mmHg. The median number of days on the ventilator was 2, with incidence of severe primary graft dysfunction in this cohort being 2.0%. The early survival (30-day or in-hospital mortality) of this group of patients was 97%. They concluded that the technique of modified reperfusion in human lung transplantation is associated with a low incidence of severe primary graft dysfunction and favorable short-term outcomes. Another study by Porteous *et al.* highlights that diastolic dysfunction also plays a role in increasing the risk of PGD development (41). These studies provide substantial evidence for the key role that flow along the endothelium has in instigating lung damage, as IRI is essential in enabling PGD to develop.

Initiation of IRI provokes endothelial cells to up-regulate various adhesion markers on their surface, produce cytokines and begin actively contributing to inflammation (42-44). ICAM-1 and PECAM-1 have both been implicated in ALI and inflammation in general, with these facilitating leukocyte transmigration from the circulation into the airways (35). ICAM-1 levels can be seen to relate to patient outcome in human studies (45). The integrins Mac-1 and $\alpha_4\beta_1$ were shown in a mouse model of lung infection by Kadioglu *et al.* to not only be essential in allowing neutrophil and T lymphocyte movement into the airways, but also facilitated the immunological response that was directed against the pathogen by these cells (37).

The other major effect that stimulation of the endothelium causes is production of cytokines by these cells. Endothelial cells have been shown to directly participate in amplifying the inflammatory response and can produce PGD-associated cytokines including PAI-1 and IL-17. These are detrimental to the function of the lung, with levels of both of these examples having been linked with worsening outcomes of PGD in patients (46-48). Activation of endothelial cells primes the environment for the secondary 'wave' of leukocyte influx into the pulmonary airways. It is this secondary phase that is ultimately so damaging.

Many key mediators released as a result of inflammation can also directly affect the structural integrity and viability of the endothelium. IRI initiates generation of caspase-8 and -9 that have been shown in a rat model to sustain mitochondrial injury and overall contribute in a

Table 2 Neutrophilic enzymes

Neutrophil secretion	Example(s)	Function(s)	Role in pathogenesis of PGD
Proteases	Neutrophil elastase, cathepsins, gelatinases, collagenases	Peptide cleavage, cytokine release	Destruction of airways and pulmonary barriers
Hydrolytic enzymes	Alkaline phosphatase, lysozymes	Digestion/breakdown of pathogenic components	Destruction of airways, reduction of oxygenation
Reactive oxygen species	Superoxide ion (O_2^-), peroxide (O_2^{2-}), tyrosyl free radical	Catalysis of pathogenic structural components	Leukocyte recruitment, tissue disruption
Pro-inflammatory cytokines	IFNγ, IL-1β, IL-6, IL-8, TNFα	Various pro-inflammatory mediators	Establishment of pathology, recruitment of immune cells, tissue disruption

detrimental manner to the consequences of reperfusion. In lung injury, apoptosis has generally been shown to play a key role in mediating significant tissue damage (49,50). Li *et al.* highlight how TNF-alpha and LPS-mediated damage *in vitro* triggers activation of MAPK pathways that culminates in substantially increased vascular endothelial permeability, translating to impaired barrier functions within an *in vivo* setting and disrupting gaseous exchange within the airways. CXCL8, which is released in large quantities by a variety of cells to attract leukocytes out of circulation has been shown *in vitro* to directly regulate cell permeability by causing down-regulation of tight junctions. This was seen to operate in a time-dependent manner, putting forward the likely scenario that accumulated CXCL8 gradually wears down the endothelial integrity over time (51). This is in turn supported by human studies which have shown that IL-8 levels in the lungs of DBD donors correlate in a concentration-dependent manner to PGD severity and mortality (52). Vascular permeability is therefore one of the major facets in lung insult and thus plays a large role in PGD pathogenesis.

Once localised to the airways, neutrophil activation contributes to the significant inflammation in this microenvironment. Multiple studies highlight how platelet-neutrophil interactions are key to enabling PMN activation in a number of autoimmune disease pathologies, including acute lung conditions (53-55). This subsequent activation is associated with production of numerous protease enzymes. Neutrophil elastase (NE) can utilise a wide range of extracellular matrix (ECM) proteins as substrates and thus disrupting airways integrity. Furthermore; release of cytokines such as IL-1β have also been attributed to the action of this enzyme. A study by Ishii *et al.* used a bilateral nephrectomy model to induce ALI and identified that application of an NE antagonist reduced levels

of cytokines such as IL-6, as well as numerous other inflammatory processes associated with this model (56). Matrix metalloproteinases (MMPs) target a wide range of substrates, with neutrophils being known to secrete these when activated. MMP-8 (neutrophil collagenase) levels in BALF were correlated with severity of disease in bronchiectasis patients, whilst another study found that blood levels of MMP-9 could be used to help differentiate between PGD grades 2/3 and individuals with no PGD present (57). These studies strongly support neutrophil degranulation being a major contributory factor to PGD disease progression.

Neutrophils are also responsible for the secretion of neutrophil extracellular traps (NETs) (58). These are strands of chromatin with the ability to bind a range of intercellular secretions and granules and ultimately ensnare extracellular pathogens. Sayah *et al.* used two experimental murine models to confirm that the presence of platelet-mediated NET formation contributed to PGD pathology. Prevention of NET formation by direct disruption with DNase I was seen to reduce the amount of lung injury present. Epidemiological evidence for this pathogenic role is provided in the study, as human BALF samples were analysed for the presence of NE-DNA complexes. Generally, these were present to a much higher degree within individuals with moderate to severe grades of PGD (59). *Table 2* outlines the enzymes secreted by activated neutrophils and the role that plays in PGD pathophysiology.

Alveolar macrophages (AMs) activation and leukocyte recruitment

AMs are a niche population that are located within the alveolar space and airways and are continuously surveying the airways for pathogen-associated molecular patterns

(PAMPs). The pro-inflammatory functions of AMs are normally tightly regulated by epithelial signals, including CD200 binding and secretions of the cytokines TGF-β and IL-10 (60). Damage to the airways caused as a result of IRI provokes an initial wave of cellular apoptosis, followed by a subsequent secondary necrosis. This releases an array of previously detailed DAMPs such as RAGE and HMGB1, with the latter in particular stimulating AMs to migrate to the site of inflammation (61). Damage to the epithelium also has the effect of decreasing the concentrations of anti-inflammatory mediators that are continuously secreted into the airways. Lack of effective cellular regulation produces populations of cells with an inflammatory profile, characterised in the case of kidney IRI by heightened cytokine production and increased oxidative burst that resembles an 'M1' polarized phenotype (62,63). Several studies have highlighted the importance of AMs in establishing IRI (64-66). A study carried out in rat lungs utilised liposomal clodronate to knockdown resident macrophage populations. This not only reduced the concentration of several known inflammatory cytokines, including; MIP-1, MIP-2α and TNFα, but also protected against much of the epithelial damage that is such a hallmark of IRI (67). Balamayooran *et al.* used an MCP-1$^{-/-}$ mouse strain to demonstrate that these mice had a significantly impaired clearance of bacteria when infected with *Escherichia coli*, caused by lower cytokine levels, less cellular adhesion and a reduced capacity of neutrophil influx. Shah *et al.* correlate this in patients, noting that levels of MCP-1 at 24 h can be used as a biomarker for PGD (45,68). Other models of lung diseases such as IPF also display a similar dependency on AMs to initiate inflammation (66). Many of these studies further demonstrate that depletion of this niche macrophage population can often alleviate much of the damage observed in these attempts to model ALI. Finally, the evidence gathered by these models is supported by *in vitro* data, as AMs have been shown to produce levels of the pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNFα when stimulated, demonstrating directly relatable proof for these mechanisms of action (69,70).

Disruption of the tissues - loss of epithelial integrity

Multiple studies identifying epithelial injury markers having a high association with more severe grades of PGD. Patel *et al.* note the detrimental effect that TNF-mediated cell death has on epithelial integrity in their 2013 study, with the presence of TNFα already being well established in the pathology of ALI in general with regard to leukocyte recruitment into the airways (71). RAGE is a major DAMP in PGD and levels have been correlated with higher grades of disease and linked to lengthened hospital stays by multiple patient studies, underlining how disruption of epithelial integrity appears to be a major factor in post-transplant failure (34,72). Expression levels were found to correlate with increased length of hospitalization by Pelaez *et al.* in a 2010 study of PGD patient BALF samples (73). RAGE is of course known to be a receptor for the aforementioned DAMP HMGB1, thus higher levels of RAGE on the surface of leukocytes enables higher levels of activation through this pathway and thus worsens the pathology of PGD. This link is confirmed in a model by Sharma *et al.* (2013) who used an *in vivo* hilar clamp model to induce IRI in C57BL/6 wild-type mice. Upon treatment with recombinant HMGB1, symptoms of IRI considerably worsened, but this was not the case when the same model was applied to RAGE$^{-/-}$ mice (74).

One notable contributory factor towards PGD is the presence of pre-transplant anti-col(V) antibodies. Iwata *et al.* identified in a 2008 study that epithelial tissue and not endothelial tissue showed expression of this, which explained why exclusively epithelial cells were targeted by an anti-col(V), CD4+ mediated cytotoxic response. Examination of patient samples identified that individuals with pre-formed antibodies had strong likeliness of developing PGD, confirming this link (75). This autoantibody response has been confirmed as playing a significant role in a murine model by Bharat *et al.* Here, the authors note that in a syngeneic transplantation model, lung-restricted antibodies directed against col(V) showed a dose-dependent response, characterised by poor oxygenation compared to the isotype controls performed (76). Pre-formed anti-col(V) antibodies in patients are therefore a significant factor in potentially damaging the epithelium in acute lung injuries.

Whilst epithelial damage is indeed an early hallmark of primary graft dysfunction evidence supports the contributory role that these cells in fact have towards worsening of pathology in many cases. One study highlighted that once produced; IL-17 and TNFα act in synergy with one another to promote the epithelial up-regulation of CXCL1, which mediates potent migration of neutrophils to the site of expression, as well as being known to modulate leukocyte functions in immunity (47,48,77). This observation was also correlated with significantly less pulmonary dysfunction when this NADPH-mediated pathway was blocked *in vivo* in a study by Sharma *et al.*, who

noted down-regulation in expression of CXCL1, as well as CCL2, CCL5 and IL-6 (78). The epithelial layers therefore are not only significant targets in IRI, but also actively support the progression of this injury to development of clinically relevant PGD.

Recent studies have started to map out the signalling pathways that lead to IRI. One such pathway involves toll-like receptors (TLRs); an important class of pattern-recognition receptor that are located in abundance on the surface of AMs. Several studies have attempted to model ALI and highlighted the importance of TLR4 in establishing ALI pathology. A study in 2012 utilised pulmonary arterial occlusion to highlight that mice could generate inflammatory mediators when exposed to a model of IRI, with this capability being abrogated if TLR4$^{-/-}$ mice were used (79). One *in vivo* model finding that using TLR4$^{-/-}$ mice was sufficient to not only reduce the amount of pro-inflammatory markers produced in the wild-type, but also prevented efficient neutrophil recruitment into the airways (79). Ding *et al.* also managed to identify that activated TLR4 can bring about activation of TLR3 via an NF-κβ-mediated pathway and that this leads to an up-regulation of PMN migration in the context of ALI (80). Further evidence for the importance of the role of TLR4 is demonstrated by Xu *et al.* who note that levels of micro RNA-21 (miR-21) have been noted as being reduced in patients with higher grades of PGD. This microRNA acts to down-regulate TLR4 expression, as well as levels of other signalling molecules, highlighting a pathway by which individuals with lower levels may be more susceptible to excess inflammation post-transplantation (81). AM populations by Dhaliwal *et al.* were seen to reduce neutrophil influx into the airways (67,79). The authors note that once this damage has been avoided, replenishment of macrophage populations is not sufficient to initiate damage to the airways, but this can be done via later administration of PMNs, effectively signalling the 'guiding' role that AMs perform within PGD and other pulmonary abnormalities. This provides evidence for the importance of AMs in modulating PMN migration in the context of ALI and subsequent development into severe grades of PGD. Lastly, TLR4 has been further implicated in PGD progression by activating NETosis in neutrophils, which also correlates with PGD grade. Knockdown of macrophages also prevented IRI development in this model, with lack of neutrophil infiltration into the airways remaining absent even after 3 h had elapsed (53). NETosis will be discussed in more depth later.

The role of CD4+ T lymphocytes in facilitating leukocyte recruitment

Lymphocytes are typically more involved in the latter stages of PGD development. Once the 'resident' AMs have detected and responded to IRI within the lungs, they can then initiate intercellular communication with donor T lymphocytes. CD4+ cells mediate these effects in PGD, and to a much lesser extent CD8+ cells. In a murine model of IRI; depletion of T lymphocytes was seen to be sufficient to alleviate the worst of the pathologically associated symptoms (82). The authors also note that depletion of CD4+ T lymphocytes within this system was sufficient to reduce the amount of PMN infiltration into the tissues. Evidence therefore points towards them having a 'chaperone' role; in that they stimulate and direct neutrophils to infiltrate the lung, which will then cause the majority of the damage to the pulmonary environment. The mechanisms of infiltration into the airways appear to be homologous with those utilised by PMNs in the case of lung disease. A combination of Th2 and Th17 responses have been identified in PGD and are synonymous with severity of disease in both cases. Levels of the cytokines IL-23 and especially IL-17 have been heavily linked with development of PGD pathology (83).

As highlighted in the section on epithelial damage; pre-formed anti col(V) antibodies are a significant factor in the outcome of a lung transplant, with immunity mediated by CD4+ T cells being identified by many studies as a significant observation within PGD. Circulating antibodies bind to these regions on epithelial cells and the exposed Fc regions will then bind to receptors on the surface of professional phagocytes, namely the AMs present in the airways. Numerous studies have documented the presence of anti-col(V) immunity present within PGD patients (75,84). This is further corroborated by the links between antibody presence and transplant outcome which again point towards a higher incidence being of great significance in development of graft injury.

Mechanisms involved in PGD progression and disease-associated biomarkers

Much of the work to identify biomarkers of PGD has come from the 'Lung Transplant Outcomes Group'. This was sponsored by the United States National Institutes of Health and collected data on lung transplant patients at 10 transplant U.S. centres between 2002 and 2010. Links have

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Table 3 Biomarkers associated with primary graft dysfunction

Biomarker/molecule	Function(s)
RAGE/sRAGE	Intracellular DNA-binding protein, pattern-recognition receptor (PRR), up-regulation of inflammatory processes, auto-inflammatory disease
IL-8/CXCL8	Neutrophil/leukocyte chemoattractant, neutrophil activation, phagocytosis
IL-17	Pro-inflammatory cytokine, monocyte/neutrophil chemoattractant, autoimmune disease, allergic reactions
Ang2	Promotes apoptosis, devascularisation, autoimmune disease
MCP-1	Monocyte/basophil chemoattractant, degranulation, tumour formation
P-selectin	Endothelial/thrombocyte adhesion marker, leukocyte recruitment, thrombocyte aggregation, tumour metastasis
ICAM-1	Endothelial/leukocyte adhesion marker, leukocyte transmigration, pro-inflammatory signalling, rhinovirus entry site
PAI-1	Inhibition of thrombolysis, promotes progression to fibrosis, inhibits action of MMPs
Col(V)	Major structural protein component of connective tissues
SP-D	Chemoattractant for alveolar macrophages and neutrophils
Protein C	Major component of anticoagulation pathway, anti-inflammatory effects, anti-apoptotic factor, promotes endothelial barrier function
Pentraxin 3	Acute phase protein, assists clearance of apoptotic cells

MMP, matrix metalloproteinase.

been made between relative levels of the proteins listed in *Table 3* and risk of PGD development. Whilst much of this information on molecular associations with outcomes in PGD remains epidemiological, recent studies have begun to attempt to map out many of the signalling pathways that lead to exacerbation of IRI. Implicated pathways within the pathogenesis involve receptors such as the toll- and NOD-like receptors (TLRs and NLRs) and up-regulation of cytokines such as IL-1β. Continued research into these pathways remains ongoing in the hope of identifying novel targets for therapeutic intervention.

Shah *et al.* measured levels of a panel of biomarkers in the plasma of a cohort of 315 PGD patients. This contained molecules involved in a range of processes such as lung epithelial damage (sRAGE), cellular adhesion (ICAM-1) and coagulation (PAI-1). All were successfully linked with PGD grade and when used in combination with one of the others and the authors conclude that addition of any one of these to the clinical PGD grading significantly improved 90-day mortality prediction (85). Another detailed analysis of patient BAL fluid samples by Cantu *et al.* in 2013 revealed a wide range of immune pathways that associated heavily with enhanced cytokine release in the case of PGD development. IL-1, IL-6 and CCL4 all showed enhanced expression levels, with signalling pathways associated with

immune receptors such as the TLRs were all expressed to higher degrees as well (26).

These aforementioned receptors become activated to release substantial amounts of pro-inflammatory mediators. Notable examples in the case of acute lung pathologies are CXCL8 and IL-17. With regard to the former, a 2001 study by Fisher *et al.* highlighted how levels of IL-8 within donor lungs could be linked to graft failure early on post-transplantation (86). As previously detailed, this cytokine plays a key role in recruiting PMNs and other leukocytes into the pulmonary airways during inflammation (87). The indispensable role of IL-17 within auto-inflammatory diseases is well-renowned and has been closely linked to PGD progression. IL-17 receptor (IL-17R) polymorphism has also been shown to display a predisposition to PGD development post-lung transplantation, suggesting a role for assisting neutrophil recruitment to the lungs (47).

Prevention and treatment of PGD

Due to a lack of appropriately powered clinical studies, there is no clear, overall consensus on the treatment of PGD after lung transplantation. Given the similar clinical features and radiographic findings seen in both PGD and ARDS, many of the potential treatment options have been extrapolated

Table 4 Selected potential treatment strategies

Mechanism of action	Examples investigated
Replacing endogenous cytoprotective substances	Prostaglandins
	Nitric oxide
	Surfactant
	Adenosine
	Endothelium-derived relaxing factor
Inhibiting pro-inflammatory mediators	Platelet activating factor 1 inhibitor
	Inhibitor of ROS
Inhibiting neutrophil and neutrophil derived mediators	Cytokines (TNF-alpha, IL-1beta)
	Proteases
	Complement cascade

ROS, reactive oxygen species.

from the management of ARDS. Strategies used to prevent and minimise the development and severity of PGD include optimising donor selection, matching and management pre-operatively; improving lung preservation and storage techniques and improving reperfusion techniques. Several therapeutic agents have been studied in an effort to reduce the incidence of PGD, with a selection of these being depicted in *Table 4*. Soluble complement receptor 1 inhibitor, plasminogen activating factor antagonist and exogenous surfactant demonstrated beneficial effects on surrogates of PGD including the A-a gradient.

Surfactant is synthesised by type 2 pneumocytes and secreted into the alveolar space where it forms a stable monolayer, resulting in reduced surface tension in the alveoli, preventing atelectasis and alveolar oedema. Experimental studies have demonstrated the link between ischaemia, cold storage and reperfusion with alterations in surfactant composition and function leading to reduction in pulmonary compliance, atelectasis, pulmonary oedema and oxygenation (88). Amital *et al.* in a prospective randomised study demonstrated that delivery of surfactant through a bronchoscope after bronchial anastomosis is established could improve oxygenation and lead to reduced PGD grade, severe PGD rates, earlier extubation and shorter ICU length of stay (89).

Recommendations have emerged for lung protective ventilation (LPV) for PGD patients based on the pre-existing evidence for this strategy in ARDS.

Veno-arterial ECMO has been used for salvage of refractory hypoxaemia due to severe PGD following lung transplantation. VA-ECMO intraoperatively instead of CPB

has been associated with shorter duration of mechanical ventilation and ICU/hospital length of stay, and lower transfusion requirements, but no statistically significant difference in 90-day mortality (90).

Normothermic EVLP is an emerging evaluation technique for high risk donor organs and allows for a period of normothermic assessment and reconditioning (47,91-96). Steen *et al.* in Lund, Sweden were the first to successful carry out EVLP in 2001 using DCD donor lungs. Cypel and colleagues then published results of their landmark study in 2011 where they reported excellent clinical outcomes using donors considered unsuitable for transplantation (97). EVLP also offers a platform through which to deliver targeted therapeutic agents to enhance the quality of the donor lungs. The results of a randomised control clinical trial using a portable extracorporeal perfusion system with the aim to reduce ischaemic time are awaited (98,99).

Conclusions

PGD has significant impact on the short and longer-term outcomes for lung transplant patients, and understanding its underlying pathophysiology is crucial to developing novel strategies aimed at identifying and reducing PGD risk. The immunological and inflammatory cellular and molecular mechanisms involved in the pathogenesis of PGD are highly complex and future experimental models are needed to investigate these further. Findings from PGD patient multicentre outcome and genetic studies are now being analysed in experimental lung transplant models with the aim to develop new therapeutic approaches.

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Footnote

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References

1. Snell GI, Yusen R, Weill D, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction Part I: Definition and Grading A 2016 consensus group statement of The International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2017. [Epub ahead of print].

2. Christie JD, Bellamy S, Ware LB, et al. Construct validity of the definition of primary graft dysfunction after lung transplantation. J Heart Lung Transplant 2010;29:1231-9.

3. Daud SA, Yusen RD, Meyers BF, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2007;175:507-13.

4. Whitson BA, Prekker ME, Herrington CS, et al. Primary graft dysfunction and long-term pulmonary function after lung transplantation. J Heart Lung Transplant 2007;26:1004-11.

5. Diamond JM, Lee JC, Kawut SM, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med 2013;187:527-34.

6. Whitson BA, Nath DS, Johnson AC, et al. Risk factors for primary graft dysfunction after lung transplantation. J Thorac Cardiovasc Surg 2006;131:73-80.

7. Christie JD, Kotloff RM, Ahya VN, et al. The effect of primary graft dysfunction on survival after lung transplantation. Am J Respir Crit Care Med 2005;171:1312-6.

8. Christie JD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. J Heart Lung Transplant 2012;31:1073-86.

9. Kreisel D, Krupnick AS, Puri V, et al. Short- and long-term outcomes of 1000 adult lung transplant recipients at a single center. J Thorac Cardiovasc Surg 2011;141:215-22.

10. Prekker ME, Nath DS, Walker AR, et al. Validation of the proposed International Society for Heart and Lung Transplantation grading system for primary graft dysfunction after lung transplantation. J Heart Lung Transplant 2006;25:371-8.

11. de Perrot M, Liu M, Waddell TK, et al. Ischemia–Reperfusion–induced Lung Injury. Am J Respir Crit Care Med 2003;167:490-511.

12. Gelman AE, Fisher AJ, Huang HJ, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction Part III: Mechanisms: A 2016 Consensus Group Statement of the International Society for Heart

and Lung Transplantation. J Heart Lung Transplant 2017. [Epub ahead of print].

13. Kuntz CL, Hadjiliadis D, Ahya VN, et al. Risk factors for early primary graft dysfunction after lung transplantation: a registry study. Clin Transplant 2009;23:819-30.

14. Krutsinger D, Reed RM, Blevins A, et al. Lung transplantation from donation after cardiocirculatory death: a systematic review and meta-analysis. J Heart Lung Transplant 2015;34:675-84.

15. Sabashnikov A, Patil NP, Popov AF, et al. Long-term results after lung transplantation using organsfrom circulatory death donors: a propensity score-matched analysis. Eur J Cardiothorac Surg 2016;49:46-53.

16. Christie JD, Kotloff RM, Pochettino A, et al. Clinical risk factors for primary graft failure following lung transplantation. Chest 2003;124:1232-41.

17. Baldwin MR, Peterson ER, Easthausen I, et al. Donor age and early graft failure after lung transplantation: a cohort study. Am J Transplant 2013;13:2685-95.

18. Liu Y, Liu Y, Su L, et al. Recipient-related clinical risk factors for primary graft dysfunction after lung transplantation: a systematic review and meta-analysis. PLoS One 2014;9:e92773.

19. Bonser RS, Taylor R, Collett D, et al. Effect of donor smoking on survival after lung transplantation: a cohort study of a prospective registry. Lancet 2012;380:747-55.

20. Lowery EM, Kuhlmann EA, Mahoney EL, et al. Heavy alcohol use in lung donors increases the risk of primary graft dysfunction. Alcohol Clin Exp Res 2014;38:2853-61.

21. Oto T, Excell L, Griffiths AP, et al. The implications of pulmonary embolism in a multiorgan donor for subsequent pulmonary, renal, and cardiac transplantation. J Heart Lung Transplant 2008;27:78-85.

22. Lederer DJ, Kawut SM, Wickersham N, et al. Obesity and primary graft dysfunction after lung transplantation: the Lung Transplant Outcomes Group Obesity Study. Am J Respir Crit Care Med 2011;184:1055-61.

23. Lederer DJ, Wilt JS, D'Ovidio F, et al. Obesity and underweight are associated with an increased risk of death after lung transplantation. Am J Respir Crit Care Med 2009;180:887-95.

24. Fang A, Studer S, Kawut SM, et al. . Elevated pulmonary artery pressure is a risk factor for primary graft dysfunction following lung transplantation for idiopathic pulmonary fibrosis. Chest 2011;139:782-7.

25. Barr ML, Kawut SM, Whelan TP, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part IV: recipient-related risk factors and

markers. J Heart Lung Transplant 2005;24:1468-82.

26. Cantu E, Lederer DJ, Meyer K, et al. Gene set enrichment analysis identifies key innate immune pathways in primary graft dysfunction after lung transplantation. Am J Transplant 2013;13:1898-904.

27. Machuca TN, Cypel M, Yeung JC, et al. Protein expression profiling predicts graft performance in clinical ex vivo lung perfusion. Ann Surg 2015;261:591-7.

28. Nagendran M, Maruthappu M, Sugand K. Should double lung transplant be performed with or without cardiopulmonary bypass? Interact Cardiovasc Thorac Surg 2011;12:799-804.

29. Szeto WY, Kreisel D, Karakousis GC, et al. Cardiopulmonary bypass for bilateral sequential lung transplantation in patients with chronic obstructive pulmonary disease without adverse effect on lung function or clinical outcome. J Thorac Cardiovasc Surg 2002;124:241-9.

30. Thabut G, Vinatier I, Stern JB, et al. Primary graft failure following lung transplantation: predictive factors of mortality. Chest 2002;121:1876-82.

31. Weber D, Cottini SR, Locher P, et al. Association of intraoperative transfusion of blood products with mortality in lung transplant recipients. Perioper Med (Lond) 2013;2:20.

32. Aeba R, Griffith BP, Kormos RL, et al. Effect of cardiopulmonary bypass on early graft dysfunction in clinical lung transplantation. Ann Thorac Surg 1994;57:715-22.

33. Entezari M, Javdan M, Antoine DJ, et al. Inhibition of extracellular HMGB1 attenuates hyperoxia-induced inflammatory acute lung injury. Redox Biol 2014;2:314-22.

34. Shah D, Romero F, Stafstrom W, et al. Extracellular ATP mediates the late phase of neutrophil recruitment to the lung in murine models of acute lung injury. Am J Physiol Lung Cell Mol Physiol 2014;306:L152-61.

35. Liu G, Place AT, Chen Z, et al. ICAM-1-activated Src and eNOS signaling increase endothelial cell surface PECAM-1 adhesivity and neutrophil transmigration. Blood 2012;120:1942-52.

36. Liu G, Vogel SM, Gao X, et al. Src phosphorylation of endothelial cell surface intercellular adhesion molecule-1 mediates neutrophil adhesion and contributes to the mechanism of lung inflammation. Arterioscler Thromb Vasc Biol 2011;31:1342-50.

37. Kadioglu A, De Filippo K, Bangert M, et al. The integrins Mac-1 and alpha4beta1 perform crucial roles in neutrophil and T cell recruitment to lungs during Streptococcus

pneumoniae infection. J Immunol 2011;186:5907-15.

38. Simms HH, D'Amico R. Increased PMN CD11b/CD18 expression following post-traumatic ARDS. J Surg Res 1991;50:362-7.

39. Todd CM, Salter BM, Murphy DM, et al. The effects of a CXCR1/CXCR2 antagonist on neutrophil migration in mild atopic asthmatic subjects. Pulm Pharmacol Ther 2016;41:34-9.

40. Schnickel GT, Ross DJ, Beygui R, et al. Modified reperfusion in clinical lung transplantation: the results of 100 consecutive cases. J Thorac Cardiovasc Surg 2006;131:218-23.

41. Porteous MK, Ky B, Kirkpatrick JN, et al. Diastolic Dysfunction Increases the Risk of Primary Graft Dysfunction after Lung Transplant. Am J Respir Crit Care Med 2016;193:1392-400.

42. Nomellini V, Brubaker AL, Mahbub S, et al. Dysregulation of neutrophil CXCR2 and pulmonary endothelial icam-1 promotes age-related pulmonary inflammation. Aging Dis 2012;3:234-47.

43. Young MR. Endothelial cells in the eyes of an immunologist. Cancer Immunol Immunother 2012;61:1609-16.

44. Zhang Y, Guan L, Yu J, et al. Pulmonary endothelial activation caused by extracellular histones contributes to neutrophil activation in acute respiratory distress syndrome. Respir Res 2016;17:155.

45. Shah RJ, Diamond JM, Lederer DJ, et al. Plasma Monocyte Chemotactic Protein-1 (MCP-1) Levels at 24 hours are a Biomarker of Primary Graft Dysfunction Following Lung Transplantation. Transl Res 2012;160:435-42.

46. Sapru A, Curley MA, Brady S, et al. Elevated PAI-1 is associated with poor clinical outcomes in pediatric patients with acute lung injury. Intensive Care Med 2010;36:157-63.

47. Somers J, Ruttens D, Verleden SE, et al. Interleukin-17 receptor polymorphism predisposes to primary graft dysfunction after lung transplantation. J Heart Lung Transplant 2015;34:941-9.

48. Song HW, Liu W, Yang C, et al. Interleukin-17A promotes paraquat-induced acute lung injury on mice. Int J Clin Exp Pathol 2017;10:2436-45.

49. Scarabelli TM, Stephanou A, Pasini E, et al. Different signaling pathways induce apoptosis in endothelial cells and cardiac myocytes during ischemia/reperfusion injury. Circ Res 2002;90:745-8.

50. Schmidt EP, Tuder RM. Role of Apoptosis in Amplifying Inflammatory Responses in Lung Diseases. J Cell Death

2010;2010:41-53.

51. Yu H, Huang X, Ma Y, et al. Interleukin-8 Regulates Endothelial Permeability by Down-regulation of Tight Junction but not Dependent on Integrins Induced Focal Adhesions. Int J Biol Sci 2013;9:966-79.

52. De Perrot M, Sekine Y, Fischer S, et al. Interleukin-8 Release during Early Reperfusion Predicts Graft Function in Human Lung Transplantation. Am J Respir Crit Care Med 2002;165:211-5.

53. Caudrillier A, Kessenbrock K, Gilliss BM, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest 2012;122:2661-71.

54. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 2007;13:463-9.

55. Kornerup KN, Salmon GP, Pitchford SC, et al. Circulating platelet-neutrophil complexes are important for subsequent neutrophil activation and migration. J Appl Physiol (1985) 2010;109:758-67.

56. Ishii T, Doi K, Okamoto K, et al. Neutrophil elastase contributes to acute lung injury induced by bilateral nephrectomy. Am J Pathol 2010;177:1665-73.

57. Craig VJ, Quintero PA, Fyfe SE, et al. Profibrotic activities for matrix metalloproteinase-8 during bleomycin-mediated lung injury. J Immunol 2013;190:4283-96.

58. Jiang S, Park DW, Tadie JM, et al. Human resistin promotes neutrophil pro-inflammatory activation, neutrophil extracellular trap formation, and increases severity of acute lung injury. J Immunol 2014;192:4795-803.

59. Sayah DM, Mallavia B, Liu F, et al. Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med 2015;191:455-63.

60. Meuth SG, Simon OJ, Gnimm A, et al. CNS inflammation and neuronal degeneration is aggravated by impaired CD200-CD200R-mediated macrophage silencing. J Neuroimmunol 2008;194:62-9.

61. Kokkola R, Andersson A, Mullins G, et al. RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. Scand J Immunol 2005;61:1-9.

62. Huen SC, Huynh L, Marlier A, et al. GM-CSF Promotes Macrophage Alternative Activation after Renal Ischemia/Reperfusion Injury. J Am Soc Nephrol 2015;26:1334-45.

63. Lee S, Huen S, Nishio H, et al. Distinct macrophage phenotypes contribute to kidney injury and repair. J Am Soc Nephrol 2011;22:317-26.

64. Tatham KC, O'Dea KP, Romano R, et al. Retention and Activation of Donor Vascular Monocytes in Transplanted Lungs Suggests a Central Role in Primary Graft Dysfunction. Am J Respir Crit Care Med 2016;193:A4890

65. Tsushima Y, Jang JH, Yamada Y, et al. The depletion of donor macrophages reduces ischaemia-reperfusion injury after mouse lung transplantation. Eur J Cardiothorac Surg 2014;45:703-9.

66. Zhang-Hoover J, Sutton A, Van Rooijen N, et al. A critical role for alveolar macrophages in elicitation of pulmonary immune fibrosis. Immunology 2000;101:501-11.

67. Dhaliwal K, Scholefield E, Ferenbach D, et al. Monocytes control second-phase neutrophil emigration in established lipopolysaccharide-induced murine lung injury. Am J Respir Crit Care Med 2012;186:514-24.

68. Balamayooran G, Batra S, Balamayooran T, et al. Monocyte chemoattractant protein 1 regulates pulmonary host defense via neutrophil recruitment during Escherichia coli infection. Infect Immun 2011;79:2567-77.

69. Ishii Y, Yang H, Sakamoto T, et al. Rat alveolar macrophage cytokine production and regulation of neutrophil recruitment following acute ozone exposure. Toxicol Appl Pharmacol 1997;147:214-23.

70. Losa García JE, Rodríguez FM, Martín de Cabo MR, et al. Evaluation of inflammatory cytokine secretion by human alveolar macrophages. Mediators Inflamm 1999;8:43-51.

71. Patel BV, Wilson MR, O'Dea KP, et al. TNF-induced death signaling triggers alveolar epithelial dysfunction in acute lung injury. J Immunol 2013;190:4274-82.

72. Scheiber-Camoretti R, Mehrotra A, Yan L, et al. Elevated S100A12 and sRAGE are associated with increased length of hospitalization after non-urgent coronary artery bypass grafting surgery. Am J Cardiovasc Dis 2013;3:85-90.

73. Pelaez A, Force SD, Gal AA, et al. Receptor for Advanced Glycation End Products in Donor Lungs Is Associated with Primary Graft Dysfunction After Lung Transplantation. American Journal of Transplantation 2010;10:900-7.

74. Sharma AK, LaPar DJ, Stone ML, et al. Receptor for advanced glycation end products (RAGE) on iNKT cells mediates lung ischemia-reperfusion injury. Am J Transplant 2013;13:2255-67.

75. Iwata T, Philipovskiy A, Fisher AJ, et al. Anti-type V collagen humoral immunity in lung transplant primary graft dysfunction. J Immunol 2008;181:5738-47.

76. Bharat A, Chiu S, Zheng Z, et al. Lung-Restricted Antibodies Mediate Primary Graft Dysfunction and Prevent Allotolerance after Murine Lung Transplantation. Am J Respir Cell Mol Biol 2016;55:532-41.

77. Yin XT, Zobell S, Jarosz JG, et al. Anti-IL-17 therapy restricts and reverses late-term corneal allorejection. J Immunol 2015;194:4029-38.

78. Sharma AK, Mulloy DP, Le LT, et al. NADPH oxidase mediates synergistic effects of IL-17 and TNF-alpha on CXCL1 expression by epithelial cells after lung ischemia-reperfusion. Am J Physiol Lung Cell Mol Physiol 2014;306:L69-79.

79. Prakash A, Mesa KR, Wilhelmsen K, et al. Alveolar macrophages and Toll-like receptor 4 mediate ventilated lung ischemia reperfusion injury in mice. Anesthesiology 2012;117:822-35.

80. Ding X, Jin S, Tong Y, et al. TLR4 signaling induces TLR3 up-regulation in alveolar macrophages during acute lung injury. Sci Rep 2017;7:34278.

81. Xu Z, Sharma M, Gelman A, et al. Significant role for microRNA-21 affecting toll-like receptor pathway in primary graft dysfunction after human lung transplantation. J Heart Lung Transplant 2017;36:331-9.

82. Yang Z, Sharma AK, Linden J, et al. CD4+ T Lymphocytes Mediate Acute Pulmonary Ischemia-Reperfusion Injury. J Thorac Cardiovasc Surg 2009;137:695-702.

83. Bobadilla JL, Love RB, Jankowska-Gan E, et al. Th-17, Monokines, Collagen Type V, and Primary Graft Dysfunction in Lung Transplantation. Am J Respir Crit Care Med 2008;177:660-8.

84. Burlingham WJ, Love RB, Jankowska-Gan E, et al. IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants. J Clin Invest 2007;117:3498-506.

85. Diamond JM, Porteous MK, Cantu E, et al. Elevated Plasma Angiopoietin-2 Levels and Primary Graft Dysfunction after Lung Transplantation. PLOS ONE 2012;7:e51932.

86. Fisher AJ, Donnelly SC, Hirani N, et al. Elevated Levels of Interleukin-8 in Donor Lungs Is Associated with Early Graft Failure after Lung Transplantation. Am J Respir Crit Care Med 2001;163:259-65.

87. Hamilton BC, Kukreja J, Ware LB, et al. Protein biomarkers associated with primary graft dysfunction following lung transplantation. Am J Physiol Lung Cell Mol Physiol 2017;312:L531-41.

88. Veldhuizen RA, Lee J, Sandler D, et al. Alterations in pulmonary surfactant composition and activity after experimental lung transplantation. Am Rev Respir Dis 1993;148:208-15.

89. Amital A, Shitrit D, Raviv Y, et al. The use of surfactant in lung transplantation. Transplantation 2008;86:1554-9.

90. Machuca TN, Collaud S, Mercier O, et al. Outcomes of intraoperative extracorporeal membrane oxygenation versus cardiopulmonary bypass for lung transplantation. J Thorac Cardiovasc Surg 2015;149:1152-7.

91. Aigner C, Slama A, Hötzenecker K, et al. Clinical ex vivo lung perfusion--pushing the limits. Am J Transplant 2012;12:1839-47.

92. Boffini M, Ricci D, Bonato R, et al. Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with ex vivo lung perfusion. Eur J Cardiothorac Surg 2014;46:789-93.

93. Fildes JE, Archer LD, Blaikley J, et al. Clinical Outcome of Patients Transplanted with Marginal Donor Lungs via Ex Vivo Lung Perfusion Compared to Standard Lung Transplantation. Transplantation 2015;99:1078-83.

94. Machuca TN, Mercier O, Collaud S, et al. Lung transplantation with donation after circulatory determination of death donors and the impact of ex vivo lung perfusion. Am J Transplant 2015;15:993-1002.

95. Wallinder A, Ricksten SE, Silverborn M, et al. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study. Eur J Cardiothorac Surg 2014;45:40-4; discussion 4-5.

96. Wigfield CH, Cypel M, Yeung J, et al. Successful emergent lung transplantation after remote ex vivo perfusion optimization and transportation of donor lungs. Am J Transplant 2012;12:2838-44.

97. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. N Engl J Med 2011;364:1431-40.

98. TransMedics. International Randomized Study of the TransMedics Organ Care System (OCS Lung) for Lung Preservation and Transplantation (INSPIRE). 2012. Available online: https://clinicaltrials.gov/ct2/show/NCT01630434

99. TransMedics. International EXPAND Lung Pivotal Trial (EXPANDLung). 2015. Available online: https://clinicaltrials.gov/ct2/show/NCT01963780

New frontiers in immunosuppression

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Abstract: Immunosuppressive therapy is arguably the most important component of medical care after lung transplantation. The goal of immunosuppression is to prevent acute and chronic rejection while maximizing patient survival and long-term allograft function. However, the benefits of immunosuppressive therapy must be balanced against the side effects and major toxicities of these medications. Immunosuppressive agents can be classified as induction agents, maintenance therapies, treatments for acute rejection and chronic rejection and antibody directed therapies. Although induction therapy remains an area of controversy in lung transplantation, it is still used in the majority of transplant centers. On the other hand, maintenance immunosuppression is less contentious; but, unfortunately, since the creation of three-drug combination therapy, including a glucocorticoid, calcineurin inhibitor and anti-metabolite, there have been relatively modest improvements in chronic maintenance immunosuppressive regimens. The presence of HLA antibodies in transplant candidates and development of *de novo* antibodies after transplantation remain a major therapeutic challenge before and after lung transplantation. In this chapter we review the medications used for induction and maintenance immunosuppression along with their efficacy and side effect profiles. We also review strategies and evidence for HLA desensitization prior to lung transplantation and management of *de novo* antibody formation after transplant. Finally, we review immune tolerance and the future of lung transplantation to limit the toxicities of conventional immunosuppressive therapy.

Keywords: Induction; maintenance; immunosuppression; desensitization; immune tolerance

Introduction

Early immunosuppression strategies after solid organ transplantation included total body irradiation, cyclophosphamide, and methotrexate (1). Predictably, myeloablative therapy prior to solid organ transplant led to major complications related to the treatment rather than rejection (1). With the discovery of 6-mercaptopurine/ azathioprine, kidney transplantation outcomes became more successful in the 1960's, and work by Dr. Thomas Starzl and others demonstrated the effectiveness of combination immunosuppressive therapy for maintaining prolonged graft survival (1-3). Unfortunately, advances in lung transplantation did not occur as quickly after Dr. James Hardy performed the first lung transplantation in the United States in 1963 (4). It was not until the advent of cyclosporine that lung transplantation began to experience prolonged graft survival (5).

Induction therapy

The first lung transplant surgeries of the modern era were performed without the use of induction therapy (6). Unfortunately, early survival remained poor with a median survival of 3.0 years from 1988 to 1991, a survival rate

which, by the turn of the century had not significantly changed (7). Recipients were plagued by high rates of early acute cellular rejection (ACR) and bronchiolitis obliterans syndrome (BOS), or late allograft failure (7,8). ACR is attributed to immune stimulation mediated by host T-cells infiltrating the allograft after activation by MHC-presentation of foreign donor antigens. The risk of ACR in the early post-transplant period may be further increased by changes in antigen presentation associated with organ retrieval and processing (9). In order to target this T-cell mediated process, some centers proposed the use of induction therapy to deplete lymphocyte stores, disrupt T-cell function and induce immune tolerance (10,11). This reflected similar practices in cardiac (12) and renal transplantation (13). Supporters cited the additional possibility that early induction would reduce the required doses of nephrotoxic calcineurin inhibitors in the early post-transplant period (14). Critics were concerned about the risks of compromising systemic immunity and consequently increasing rates of infection and malignancy, both of which had been reported in other solid organ transplants (15,16).

While the debate over the risks and benefits of induction therapy continues, induction therapy use has increased. Induction therapy was utilized in approximately 50% of lung transplants in 2004 as compared to its use in greater than 70% of lung transplants in the first half of 2016 (17-19). Despite nearly 20 years since the first use of induction therapy, its role in lung transplantation has not been rigorously studied. A 2013 meta-analysis found only three randomized clinical trials directly addressing this question (total n=140) and demonstrated no significant difference in mortality, ACR grade 2 or higher, or BOS, though cumulative hazard ratios trended in favor of induction therapy (20). A recent retrospective analysis of the United Network for Organ Sharing (UNOS) database including more than 6,000 subjects demonstrated significantly improved survival for subjects receiving alemtuzumab or basiliximab compared to no induction therapy (21). Perhaps the tides are finally turning.

The three induction agents currently in use are polyclonal anti-thymocyte globulin (ATG), anti-CD52 monoclonal antibody (alemtuzumab) and interleukin-2 (IL-2) receptor antagonists (basiliximab). Since 2004 more than 80% of induction therapy recipients received IL-2 receptor antagonists, a continued trend upward from approximately 40% in 2006 (19).

ATG

ATG is composed of polyclonal immunoglobulins derived from either horse or rabbit exposure to human thymocytes. The resulting polyclonal immunoglobulins are directed at multiple different human lymphocyte antigens. Immunoglobulin binding leads to complement-mediated lymphocyte cell lysis, antibody-mediated cell lysis, macrophage-mediated phagocytosis and lymphocyte opsonization followed by removal through the reticuloendothelial system (22). Induction doses of ATG vary based on the given formulation (rabbit *vs.* equine) but are administered for 3 consecutive days following transplantation (10). Lymphocyte depletion may last as long as 6–8 months (23).

One of the major concerns regarding ATG use is the possibility of an acute cytokine storm in response to ATG infusion. Subjects may develop non-cardiogenic pulmonary edema, chest pain and shortness of breath. Milder syndromes have also been reported including a serum sickness-like illness with diffuse rash, fever, pruritus, myalgia and arthralgia. Serum sickness may occur days to weeks after infusion. A majority of ATG-treated patients will develop anti-rabbit or anti-equine antibodies (24) which may complicate subsequent ATG dosing. Additionally, thymoglobulin is a treatment option for high grade and/or refractory acute rejection (25) thus prior exposure to ATG may theoretically complicate subsequent use though there is no available literature to support this.

Alemtuzumab

Alemtuzumab is a monoclonal antibody directed against the cell surface marker CD52. CD52 is expressed on the surface of B-cells, T-cells, monocytes, macrophages, and NK cells (26). Alemtuzumab binds to this cell surface protein leading to complement-mediated cytolysis, antibody-mediated cytotoxicity and programmed cell death. Alemtuzumab is dosed at 30 mg IV prior to reperfusion or immediately following transplantation (27,28). Alemtuzumab has a 12-day half-life; however cell function is impaired significantly longer with monocyte, B-cell and T-cell recovery at 3, 6 and 12 months respectively (29).

Given the prolonged lymphopenia associated with alemtuzumab therapy, there is significant concern regarding risk of infection and post-transplant lymphoproliferative disease (PTLD). Recent work has been limited to single center and large registry studies and the results have been

Table 1 Review of induction therapies

Agent	Formulations (brand name)	Mechanism of action	Side effects	Percent of transplant centers using for induction*
Anti-thymocyte globulin	Horse (ATGAM); rabbit (rATG)	Polyclonal equine or rabbit antibodies directed at thymocyte cell surface proteins leading to degradation and ultimately T-cell death/depletion (22)	Acute cytokine storm; delayed serum sickness-like syndrome (24)	13% (19)
IL-2 receptor antagonist	Basiliximab (Simulect)	Binds to CD25 surface protein on T-cells thereby impairing IL-2 dependent signaling (31)	No infusion reactions (30)	76% (19)
Anti-CD52	Alemtuzumab (Campath)	Binds to CD52, expressed by B cells, T-cells, NK cells, monocytes and leads to cell lysis, prolonged leukocyte depletion (26)	Cytokine storm; prolonged lymphopenia (29)	11% (19)

*, values indicate rates of use among recipients who received induction therapy between 2004 and 2016, according to the 2017 report of the International Society for Heart and Lung Transplantation (19). ATG, anti-thymocyte globulin.

heterogeneous with multiple single center studies showing no significant difference in rates of infection (27,30) or PTLD (28) between alemtuzumab and basiliximab groups, while the UNOS registry analysis demonstrated increased rates of non-CMV infection and PTLD following the use of alemtuzumab compared to either basiliximab or no induction therapy (21).

IL-2 receptor antagonists

IL-2 receptor antagonists are chimeric antibodies (mouse + human) directed against the alpha subunit of the CD25 cell surface protein (the IL-2 receptor). Basiliximab is currently in use within the US, while daclizumab is no longer on the market due to decreased demand. These IL-2 receptor antagonists bind to the IL-2 receptor and block IL-2 dependent signaling. T-cells rely on IL-2 signaling for proliferation and differentiation thus IL-2 receptor antagonists inhibit this process. Unlike the other induction agents however, IL-2 receptor antagonists do not lead to cell death or significant T-cell depletion. Basiliximab is dosed at 20 mg at time 0 and 4 days after transplantation based on literature demonstrating increased rates of ACR if the first dose is given post-transplantation (31) mirroring findings seen in pediatric heart transplantation (32). Basiliximab has a half-life of 7.2±3.2 days (Basiliximab drug insert) but may block the receptor for up to 59±17 days when combined with triple drug therapy consisting of cyclosporine, prednisone and mycophenolate mofetil (Basiliximab drug insert). Basiliximab is a humanized antibody and thus not associated with the infusion reactions observed during ATG

and alemtuzumab treatment.

Outcomes with induction therapy

There are limited randomized controlled trials comparing no induction, ATG, alemtuzumab and basiliximab, and hence clinical practice is based largely on observational studies, large registry analyses and center preference (*Table 1*). Compared to no induction, ATG has been associated with decreased rates of ACR, increased malignancy, a trend towards increased CMV infection and no significant difference in BOS or overall graft survival (10,33). Compared to historical controls who did not receive induction, the IL-2 receptor antagonists have been associated with decreased early ACR (34,35) without increased incidence of infection or malignancy while alemtuzumab use as induction therapy has revealed more conflicting results. One large retrospective analysis showed significantly fewer episodes of ACR, greater freedom from BOS, increased overall survival with significantly fewer deaths from graft failure and a trend towards increased risk of PTLD with alemtuzumab compared to no induction (28). However, another smaller study using historical controls demonstrated no significant difference in ACR, survival or infection rates (36). Notably both studies reported reduced dose maintenance immunosuppression post-transplantation among subjects using alemtuzumab, further emphasizing the potential effectiveness of this therapy.

In head to head studies, alemtuzumab or basiliximab may be associated with lower rates of ACR and improved overall survival compared to ATG preparations (20,27,37);

though only alemtuzumab has been demonstrated to offer the additional benefit of decreased maintenance immunosuppression (38). A recent analysis of the UNOS national registry demonstrated increased survival free of BOS for subjects receiving alemtuzumab compared to basiliximab (21). Inherent in this analysis were the challenges of large observational datasets including significant differences between groups. Nonetheless, smaller observational studies have revealed similar findings (28,30). Ongoing concerns regarding the risk of prolonged lymphocyte depletion associated with alemtuzumab therapy, combined with the relatively mild side effect profile of basiliximab likely account for the growing prevalence of basiliximab use although studies addressing this question have demonstrated mixed results (11,21,30). Additional randomized controlled trials comparing rates of ACR, BOS, infection and PTLD between alemtuzumab and basiliximab are certainly warranted.

While the use of induction immunosuppression is not yet universal in lung transplantation, it is becoming increasingly common. The preponderance of evidence suggests that it is associated with lower rates of ACR and improved overall survival. Nonetheless, it is prudent for all centers to continue to consider the possibility of increased infectious and malignant complications associated with the use of some induction therapies. Further research is required to identify the optimal therapy choice and the patient population that is most likely to benefit from induction therapy.

Maintenance immunosuppression

Maintenance immunosuppressive therapy is arguably the most important part of medical care after lung transplantation, and its main purpose is to prevent acute and chronic rejection. However, this goal must be balanced with side effects and major toxicities associated with these medications. Maintenance immunosuppressive regimens for lung transplantation are largely extrapolated from observational studies and trials in renal, liver and heart transplantation (22,39). Protocols for maintenance immunosuppressive regimens vary among lung transplant centers, but typical regimens consist of a three-drug combination including a calcineurin inhibitor, an anti-metabolite and a glucocorticoid (39-42). Combination regimens allow for higher levels of immunosuppression while also minimizing the toxicities of an individual medication (22). Due to higher rates of ACR in the early post-transplant course, transplant protocols typically utilize greater intensity of immunosuppression in

the first year after transplant (43). Naturally, this potential benefit of higher intensity of immunosuppressive treatment must be balanced against the adverse effects of over-immunosuppression including greater susceptibility to infection (44) and malignancy (45).

Glucocorticoids

Glucocorticoids are a mainstay of immunosuppressive regimens after lung transplantation because they have widespread inhibitory effects on the immune system and act through a variety of signaling pathways (46). Glucocorticoids bind to the intracellular glucocorticoid receptor generating a complex that blocks the transcription of inflammatory cytokines mainly through an interaction with nuclear factor-kappa-B (NF-KB), but also through the induction of anti-inflammatory proteins such as annexin-1 and MAPK phosphatase-1 (46). Through these pathways glucocorticoids inhibit macrophage activation and reduce lymphocyte proliferation and migration (47). Glucocorticoids are certainly not without side effects; long term glucocorticoid use can lead to infectious complications, osteoporosis, diabetes, hyperlipidemia, cataracts, psychiatric and mood changes, weight gain, myopathy, hypertension and impaired wound healing (48,49). Fortunately, these risks are dose dependent and can be somewhat mitigated by reduced dosages (50). Prednisone is the classic glucocorticoid used after lung transplant; usually after an induction dose, it is reduced to 0.5 mg/kg/day in the immediate post-transplant period followed by a reduction over the next several months to a dose of 5–10 mg for maintenance treatment (39,41). Total glucocorticoid withdrawal is rare (42); a few small studies have reported varying success after withdrawal (51,52).

Calcineurin inhibitors

Cyclosporine was originally isolated from the fungus, *Tolypocladium inflatum*, and its identification as an immunosuppressant agent revolutionized solid organ transplantation (41). After the discovery of cyclosporine, the field of lung transplantation started to demonstrate notable success (5). Cyclosporine binds cyclophilins present inside cells forming a drug-receptor complex that competitively binds and inhibits calcineurin, a calcium and calmodulin-dependent protein phosphatase, resulting in the inhibition of nuclear factor of activated T-cells (NF-AT) related transcription factors (53). This leads to reduced transcriptional activation of cytokines IL-2, tumor necrosis

factor-α, IL-3, IL-4, CD40L, granulocyte-macrophage colony stimulating factor, and interferon-γ, thereby reducing T-cell activation and proliferation (54).

Cyclosporine is available in an unmodified and modified form. The unmodified form is an oil-based emulsion that has unreliable absorption (Sandimmune, Novartis Pharmaceuticals Corp.). The unmodified preparation has largely been replaced by the modified form (Neoral, Novartis Pharmaceuticals Corp.) which provides greater bioavailability and more predictable absorption (39,41). Careful therapeutic monitoring is needed to ensure efficacy and avoid toxicity. Measurements of area under the curve (AUC) have shown some superiority for cyclosporine level monitoring with reduced ACR and lower nephrotoxicity in renal transplant (55). However, determining the AUC involves multiple measurements and as a result it is quite difficult and not practical (39,41,56). Other options include measuring levels 2 hours after the dose (C2) or trough levels (C0). Studies have suggested that C2 monitoring is more closely correlated with AUC and may reduce nephrotoxicity compared with C0 monitoring (57,58). Target cyclosporine levels are dependent on the time from transplant and center specific protocols, but generally target C0 levels range from 100–450 ng/mL and C2 levels range from 800–1,400 ng/mL (39,41). Nephrotoxicity, both acute and chronic, is the principal side effect of cyclosporine and can be mitigated by reducing the target level (59). Other side effects include hirsutism, gingival hyperplasia, neurotoxicity such as seizures and tremors, hypertension, diabetes, and dyslipidemia (60-65).

Tacrolimus is another calcineurin inhibitor that was first identified in a soil sample in 1984 and became available for clinical use in 1994 after demonstrating efficacy in renal and liver transplantation and as a rescue medication in the setting of rejection (66). Tacrolimus binds to intracellular FKBP12, also forming a drug receptor complex that competitively binds with calcineurin and acts through the same pathway as cyclosporine to inhibit T-cell activation and proliferation (53). Tacrolimus is considerably more potent than cyclosporine and has oral bioavailability of around 20–25% (67). Transplant centers typically monitor trough levels despite studies that have demonstrated that post dose levels are a better predictor of AUC (68,69). Target trough levels for tacrolimus range between 5 and 15 depending on comorbidities, time from transplant and center specific protocols (39,41). The side effect profile for tacrolimus is similar to cyclosporine with less hypertension (65) and hyperlipidemia (64) but more neurotoxicity (70,71)

and diabetes (64). Both tacrolimus and cyclosporine are metabolized through the CYP450 3A4 pathway, and careful attention with dose adjustment is needed when using medications that induce or inhibit these enzymes (69).

After clinical trials demonstrated the efficacy of tacrolimus in liver and kidney transplantation, Keenan *et al.* published the first randomized controlled trial in 1995 of 133 lung transplant recipients. They compared a regimen of tacrolimus, azathioprine and prednisone, with a regimen of cyclosporine, azathioprine, and prednisone and found similar ACR rates as well as survival rates, but less obliterative bronchiolitis (OB) on transbronchial biopsies (72). Subsequent trials by Zuckerman *et al.* (73,74), Hachem *et al.* (75), and Treede *et al.* (76) reported variable results which are summarized in *Table 2*. In the largest randomized, multicenter study of 249 lung transplant recipients, Treede *et al.* demonstrated lower rates of BOS at 3 years in patients receiving a tacrolimus-based regimen compared with a cyclosporine-based regimen, 11.6% *vs.* 21.3%, respectively (P=0.037) (76). Partially as a result of these studies as well as data from other solid organ transplantation, tacrolimus currently is the preferred calcineurin inhibitor and in 2016 it was used in over 90% of lung transplant recipients at the 1-year follow-up after transplant (19,78).

Antimetabolites

Azathioprine was the first immunosuppressant in solid organ transplantation to be utilized in a multi-drug cocktail and has been in use in solid organ transplantation since the 1960's (1,79). Azathioprine is converted to 6-mercaptopurine (6-MP) and then into 6-thiouric acid, 6-methyl-MP and 6-thioguanine, ultimately acting as a nucleotide blocking agent and halting DNA replication (79). Azathioprine is usually dosed at 1 to 2 mg/kg daily with dose reductions made based on cell counts (39,41). About 10% of the population has a thiopurine methyltransferase (TPMT) deficiency which is an important enzyme in azathioprine metabolism pathway. TPMT-deficient patients can develop severe myelosuppression due to azathioprine (79). Other side effects associated with azathioprine therapy include hepatotoxicity, cholestasis and pancreatitis (39,79).

Mycophenolate is another antimetabolite; it has become the preferred anti-metabolite and in 2016, it was used in about 80% of lung transplant recipients at the 1-year follow-up after transplant (19,78). Mycophenolate is also derived from fungi and is rapidly converted to its active form mycophenolic acid (MPA) in the liver. MPA inhibits

Table 2 Tacrolimus *vs.* cyclosporine for lung transplant recipients

Study	Population, n	Methodology	Outcomes
Keenan *et al.*, 1995 (72)	133	Prospective, randomized, single center; comparing TAC/AZA/CS and CsA/AZA/CS; at time of transplant; follow-up 254–1,555 days	AR: 0.85 (TAC) *vs.* 1.09 (CsA) AR per 100 days, P=0.07; survival: 1 and 2-year: 83%, 76% in TAC *vs.* 71%, 66% in CsA group, P= NS; OB: 21.7% in TAC *vs.* 38% in CsA group, P=0.025
Zuckerman *et al.*, 2003 (73,74,77)	74	Prospective, randomized, two center, open label; comparing TAC/MMF/CS *vs.* CsA/MMF/CS; at time of transplant, mean follow-up 507 days; with later follow-up of 36 months	Survival: 1 year: 71% (TAC) *vs.* 82% (CsA), P= NS; survival: 3-year: 68% (TAC) *vs.* 57% (CsA), P=0.748; AR: 0.22 (TAC) *vs.* 0.32 (CsA) AR episodes per 100 days, P=0.097; Freedom from AR at 1 year: 46% (TAC) *vs.* 35% (CsA), P=0.774; BOS incidence: 10% *vs.* 41%, P<0.01
Hachem, *et al.*, 2007 (75)	90	Prospective, randomized, single center, open label; trial comparing TAC/AZA/CS *vs.* CsA/AZA/CS; at time of transplant, median follow-up 2.17 years	Composite end point: BOS 0p or cumulative acute rejection score or A3 or higher or cumulative lymphocytic bronchiolitis of B4 or higher; primary end point: 54.5% (TAC) *vs.* 84.8% (CsA), P=0.002; freedom from BOS 0p: not different, P=0.1
Treede, *et al.*, 2012 (76)	249	Prospective, randomized, multicenter, open label, comparing TAC/MMF/CS *vs.* CsA/MMF/CS; at time of transplant, follow-up 3 years	BOS: 11.6% (Tac) *vs.* 21.3% (CsA), P=0.037; HR of BOS: 1.97 for CsA compared with TAC; survival: 1 year: 84.6% (TAC) *vs.* 88.6% (CsA); survival: 3-year: 78.7% (TAC) *vs.* 82.8% (CsA), P=0.382; AR: no difference in cumulative rate of AR at 3 years, P=0.43

AR, acute rejection; CsA, cyclosporine; TAC, tacrolimus; MMF, mycophenolate mofetil; AZA, azathioprine; CS, corticosteroids; NS, non-significant and not reported in article; OB, obliterative bronchiolitis on transbronchial biopsy; BOS, bronchiolitis obliterans syndrome; HR, hazard ratio.

inosine monophosphate dehydrogenase, the enzyme responsible for the synthesis of guanosine nucleotides, thereby halting DNA synthesis (39,41,79). Uniquely, this is specific for B and T-cell proliferation because most cells can access salvage pathways for guanosine nucleotide synthesis, whereas B- and T-cells rely solely on the inosine monophosphate pathway (39,41,79). The most worrisome adverse effect of mycophenolate is bone marrow suppression, however diarrhea, nausea, and abdominal discomfort are common and for some, debilitating (39,79). A newer formulation of mycophenolate sodium, Myfortic, contains MPA in a delayed release capsule that may reduce GI side effects and has shown comparable efficacy in renal and heart transplantation (41). Therapeutic monitoring of mycophenolate is possible but the efficacy and importance of monitoring has not been established (39).

Mycophenolate has demonstrated superiority in preventing ACR in several randomized trials in renal, heart and liver transplantation (80-82), however, the data has been less robust in lung transplantation. A few small non-randomized studies demonstrated reduced episodes of acute allograft rejection with mycophenolate compared with azathioprine (83,84). Two larger randomized controlled trials in lung transplant recipients failed to show a reduction in the development of ACR, infection, or BOS (85,86).

These trials are summarized in *Table 3*.

However, recent analysis from the International Society for Heart and Lung Transplant (ISHLT) registry demonstrated that 38.1% of lung transplant recipients receiving mycophenolate mofetil, cyclosporine, and a corticosteroid experienced rejection within the first year compared with 58.1% of lung transplant recipients receiving azathioprine, cyclosporine and corticosteroid therapy (P<0.05) (78).

Mammalian target of rapamycin (mTOR) inhibitors

Sirolimus and everolimus are mTOR inhibitors that are structurally similar to calcineurin inhibitors and act by binding FKBP12 to form a drug-protein complex like tacrolimus. However, they instead block the mTOR instead of calcineurin, halting DNA synthesis and consequently the proliferation of T and B cells (39,79). Target trough levels for sirolimus and everolimus range from 5–15 (39,79) and 3–8 ng/mL (87,88), respectively. The side effect profiles for sirolimus and everolimus are similar including myelosuppression, diarrhea, mouth ulcers, hyperlipidemia, refractory edema, and most importantly impaired wound healing (87,88). Likely related to the effects of mTOR inhibitors on fibroblasts, endothelial

Table 3 Mycophenolate *vs.* azathioprine for lung transplant recipients

Study	Population	Methodology	Outcomes
Zuckerman *et al.*, 1999 (83)	38	Prospective cohort study compared with historical control group; MMF/CsA/CS *vs.* AZA/CsA/CS; enrolled at time of transplant, follow-up 6 months	AR: 0.29 (MMF) *vs.* 1.53 (AZA), P<0.01; Infections: 1.57 (MMF) *vs.* 2.29 (AZA), P= NS
Palmer *et al.*, 2001 (85)	81	Prospective, randomized, two center, open label; MMF/CsA/CS *vs.* AZA/CsA/CS; enrolled at time of transplant, 6 months follow-up	AR: 63% (MMF) *vs.* 58% (AZA), P=0.82; Survival: 86% (MMF) *vs.* 82% AZA, P=0.57
McNeil *et al.*, 2006 (86)	320	Prospective, randomized, multicenter trial, open label; MMF/CsA/CS *vs.* AZA/CsA/CS; enrolled at time of transplant, 3 years follow-up	AR: 56.6% (MMF) *vs.* 60.3% (AZA), P=NS; no difference in time to first rejection; survival: 1 year: 88% (MMF) *vs.* 80% (AZA), P=0.07; survival: 3-year: 75% (MMF) *vs.* 69% (AZA), P=0.18; Freedom from BOS at 3 years: 73% (MMF) *vs.* 75% (AZA), P=0.70

AR, acute rejection; CsA, cyclosporine; Tac, tacrolimus; MMF, mycophenolate mofetil; AZA, azathioprine; NS, non-significant and not reported in article; CS, corticosteroids; AE, adverse events.

cells and smooth muscle cells, early studies with sirolimus reported airway anastomotic dehiscence (89,90). As a result, subsequent studies have avoided mTOR inhibitors until at least 4 weeks after lung transplantation (87,88). Other notable potential risks with sirolimus are venous thromboembolism (91) and drug induced pneumonitis (92). Nevertheless, mTOR inhibitors have demonstrated advantages when added to immunosuppressive regimens, including allowing the reduction of target calcineurin trough levels resulting in less nephrotoxicity (93,94) and reduced risk of malignancy (95).

Although both sirolimus and everolimus have shown some efficacy in preventing rejection and supplementing immunosuppressive regimens to limit toxicity from calcineurin inhibitors, randomized trials have failed to demonstrate a clear reduction in BOS, ACR or improved survival (87,88,96). There are four randomized trials that have shown mixed results using everolimus or sirolimus in terms of reducing the incidence of allograft dysfunction. Notably three of the four trials showed less CMV infection in patients on mTOR inhibitors instead of the antimetabolites (87,88,96,97). Details of these studies are shown in *Table 4*.

Antibody mediated therapies

Immunosuppression is largely targeted at limiting cell-mediated immunity, however, the dangers of antibody mediated rejection (AMR) for the allograft have become increasingly clear over time. Unfortunately, the diagnosis of AMR in lung transplant recipients remains somewhat elusive despite the recent consensus statement by the ISHLT (98). Therapy for antibody-mediated rejection involves plasmapheresis, intravenous immunoglobulin (IVIG), rituximab, bortezomib and the newer agent carfilzomib. Plasmapheresis removes circulating antibodies and hopefully donor specific antibodies (DSA) that could target the allograft. IVIG has a combination of effects resulting in apoptosis of B cells and inhibition of the antibody-mediated complement pathway (99). Rituximab is a monoclonal anti-CD20 antibody specific for B-cells that causes apoptosis and ultimately B-cell depletion (39). Bortezomib and carfilzomib are proteasome inhibitors that cause plasma cell apoptosis (100).

Development of *de novo* DSA after transplantation is associated with poorer survival and greater risk of BOS and chronic lung allograft dysfunction (101-103). Hachem *et al.* conducted a prospective observational study of a treatment protocol for patients with *de novo* DSA in the absence of allograft dysfunction, involving rituximab with IVIG versus IVIG alone. In this study both treatments similarly cleared DSA, but patients who did not clear their DSA were more likely to develop BOS (P=0.03) (104). Although the findings from this study suggest that aggressive multi-targeted therapy to reduce DSA after transplant is beneficial even in the absence of allograft dysfunction, larger randomized trials are needed to determine how to best approach *de novo* DSA.

Table 4 mTOR inhibitors

Study	Population	Methodology	Outcomes
Snell et al., 2006 (97)	213	Prospective, randomized, multicenter, double-blind; EVE/CsA/CS vs. AZA/CsA/CS; enrolled BOS free patients at 3–36 months after transplant; 12–24 months follow-up	Efficacy failure: decline in FEV1 >15%, graft loss, death or loss to follow up: EF: 1 year: 21.8% (EVE) vs. 33.9% (AZA), P=0.046; EF: 2-year: 43.6% (EVE) vs. 44.6% (AZA), P=0.874; Decline in FEV1 >15%: 15.8% (EVE) vs. 27.7% (AZA), P=0.034; BOS: 1 year: 14.9% (EVE) vs. 24.1% (AZA), P=0.085; BOS: 2-year: 31.7% (EVE) vs. 35.7% (AZA), P=0.534; AR: 1 year: 7.9% (EVE) vs. 32.1% (AZA), P<0.001; AR: 2-year: 14.9% (EVE) vs. 41.1% (AZA), P<0.001
Bhorade et al., 2011 (96)	181	Prospective, multicenter, randomized, open label; SIR/TAC/CS vs. AZA/TAC/CS, enrolled 90 days after transplant, follow-up 36 months	No difference in AR episodes; AR: 1 year: 39% (SIR) vs. 48% (AZA), P=0.82; AR: 3-year: 46% (SIR) vs. 49% (AZA), P=0.57; no difference in BOS at year 1 or 3; BOS: 1 year: 11% (SIR) vs. 3% (AZA), P=0.11; BOS: 3-year: 30% (SIR) vs. 22% (AZA), P=0.48
Glanville et al., 2015 (87)	165	Prospective, multicenter, randomized, open-label, MPS/CsA/CS vs. RAD/CsA/CS, enrolled 1–3 months after transplant, follow-up 3 years	As per ITT analysis, no difference in BOS, survival, or time to AR, but more AR episodes in MPS group; survival: 3-year: 76% (RAD) vs. 84% (MPS), P=0.19; freedom from BOS at 3 years: 71% (RAD) vs. 70% (MPS), P=0.95; mean number of AR episodes per patient higher in MPS group, P=0.04
Strueber et al., 2016 (88)	190	Prospective, randomized, single-center; comparing EVE/CsA/CS vs. MMF/CsA/CS; enrolled 28 days after transplant with follow-up 24 months	Results below are for ITT analysis, no difference in survival, BOS incidence; high rates of cross-over and different results in per protocol analysis; survival: 2-year: 89% (EVE) vs. 87% (MMF), P=0.664; BOS incidence: 14% (EVE) vs. 21% (MMF), P=0.250

EF, efficacy failure; AR, acute rejection; CsA, cyclosporine; TAC, tacrolimus; MMF, mycophenolate mofetil; AZA, azathioprine; NS, non-significant and not reported in article; CS, corticosteroids; AE, adverse events; ITT, intention to treat; MPS, mycophenolate sodium; RAD, delayed-onset everolimus; mTOR, mammalian target of rapamycin.

Desensitization

The pre-transplant presence of anti-HLA antibodies in lung transplant candidates is being increasingly recognized and poses major challenges to patient selection and donor identification. The solid phase single antigen bead technology has increased the recognition and identification of anti-HLA antibodies, however there is significant controversy on how to approach patients with these antibodies (105). Studies have shown that pre-transplant sensitization with anti-HLA antibodies may be associated with decreased survival after transplantation (106). One management approach is to immediately label anti-HLA antibodies present above a certain threshold as unacceptable antigens for the patient and thus limit the available donor pool. Other approaches include desensitization prior to lung transplantation to attempt to reduce the anti-HLA antibody levels prior to transplant (107), and peri-operative desensitization at the time of transplantation (108).

Snyder and colleagues reported the outcomes of a desensitization protocol for highly sensitized pre-transplant patients with calculated panel reactive antibody (cPRA) ≥80%. The protocol included rituximab on day 1 and 26, plasmapheresis for seven treatments over 19 days, IV solumedrol for four doses and four doses of subcutaneous bortezomib (107). Eighteen patients started the treatment and nine patients completed the treatment with early completion due to transplant. The cPRA did not change with the protocol and the median fluorescence intensity (MFI) significantly decreased only for anti-HLA antibodies in the 5,000–10,000 MFI range but not high MFI >10,000 or low MFI <5,000 anti-HLA antibodies. This desensitization protocol was not markedly successful at broadening the donor pool, and the clinical impact of these pre-transplant anti-HLA antibodies on post-transplant outcomes remains unknown (107).

Tinckam and colleagues reported their single center experience with a perioperative desensitization strategy for patients with DSA and cPRA ≥30% (108). To avoid the toxicities of pre-transplant desensitization before transplant and to circumvent limiting the donor pool, these patients received perioperative plasmapheresis, IVIG,

ATG and MPA. Unsensitized patients were treated with the standard cyclosporine, azathioprine and prednisone regimen without ATG. One-year graft survival was nearly identical comparing sensitized patients who have DSA with unsensitized patients who did not have DSA (89% *vs.* 86%, P=0.47). This study demonstrates excellent outcomes with a perioperative desensitization approach that does not diminish the available donor pool for patients (108).

The future of immunosuppression

Survival in lung transplantation remains quite poor compared to other solid organ transplants with a median survival of 5.8 years compared to 11.9 years for heart transplantation (18,109). Early mortality is largely attributable to infection and graft failure while later mortality is due to chronic lung allograft dysfunction generally resulting from allograft rejection (18). Further complicating immunosuppression in lung transplantation, is the fact that the mechanisms of immune responses in lungs vary from those observed in other organs (110,111). With the exception of the small bowel, the lung is the only transplanted organ that is constantly exposed to the surrounding environment. Thus the immunosuppression required to avoid lung allograft rejection remains especially complicated. There are, however, promising avenues of active research to address these challenges.

Transplant immune tolerance

The major goal of post-transplant immunomodulation is to achieve transplant immune tolerance; a state in which the recipient's immune system no longer responds to the allograft as foreign. Transplant immune tolerance would obviate the need for immunosuppression while maintaining an intact immune system capable of fighting infection and surveilling for malignancy thus removing significant causes of morbidity and mortality after transplantation. Could this be possible?

Mixed chimerism is a state in which the donor and recipient hematopoietic cells co-exist creating donor-specific tolerance in the recipient. The first solid organ transplants to utilize mixed chimerism were performed in subjects who had undergone hematopoietic stem cell transplantation (HSCT) and subsequently developed renal failure requiring renal transplantation. These renal transplants were performed from the same HLA-identical donor, and subjects

did not require subsequent immunosuppression (112,113). Following the identification of this unique state of donor-specific tolerance, combined bone marrow and renal transplantation was successfully performed in subjects with multiple myeloma and associated renal failure (114,115). One of the major challenges in these approaches, has been determining the least toxic conditioning regimen possible to allow for bone marrow engraftment and to achieve mixed chimerism. Such transplants were initially performed with myeloablative chemotherapy (114) but subsequently with non-myeloablative regimens (115). Regimens used for primary renal transplantation in the absence of myeloma or other malignancy, have consisted of total lymphoid irradiation and ATG (116-118), or cyclophosphamide, anti-CD2 antibody, cyclosporine and thymic irradiation (119). Many subjects in these studies were able to completely stop all immunosuppressive therapy.

Applying this approach to lung transplantation is challenging as the conditioning regimens used in combined HSCT-renal transplantation must begin many days prior to solid organ transplantation thus complicating the unpredictable deceased donor availability and transplantation process that accounts for the vast majority of lung transplant surgeries. Studies performing HSCT after solid organ transplantation have been successful in non-human primates (120,121). A single case report in a patient with advanced lung disease from a primary immune deficiency who underwent deceased donor lung transplantation, reports successful mixed chimerism and long-term immunosuppression withdrawal with donor-derived HSCT performed 3 months after lung transplantation (122).

While work in renal transplantation has produced remarkable advances in transplant immunology, it remains prudent to consider whether renal transplantation techniques will be easily translated to lung transplantation. Studies performed in swine models of combined heart and kidney transplantation have demonstrated that co-transplantation of a kidney is protective, and removal of the renal allograft results in acute rejection of the heart (123,124). Similarly, there are numerous cases of sustained immune tolerance after withdrawal of immunosuppression in both renal and liver transplantation (125-127), while this has not been seen in lung transplantation. Additionally, the lung remains an immunologically distinct organ (110,111), and in contrast to the kidney, is constantly exposed to the surrounding environment. Unfortunately, this suggests

that the renal transplantation techniques may not be easily translated to lung transplantation.

While establishing immune tolerance is likely to be the best long-term goal for solid organ transplantation, it is likely to be many years away.

Existing medications, new applications

Bortezomib and carfilzomib are two proteasome inhibitors initially developed for the treatment of multiple myeloma. Plasma cells typically express large numbers of proteasomes which serve to ubiquinate and destroy dysfunctional or misfolded proteins. Proteasome inhibitors allow these ubiquitinated proteins to accumulate, resulting in cell death. Studies of these medications in the treatment of antibody-mediated rejection are small, but suggest they may result in significant decreases in antibody levels (128,129), clinical improvement (130) and return of pulmonary function to pre-AMR levels (100). One major area of controversy is the definitive diagnosis of AMR. Since its burden in lung transplant rejection is not well known, it is difficult to measure treatment efficacy (98).

Belatacept is a protein that binds the CD80 and CD86 receptors on antigen presenting cells (APC); these receptors are necessary for APC-mediated stimulation of T-cells. Belatacept blocks T-cell co-stimulation, cytokine production and T-cell proliferation. Belatacept was originally approved for use in renal transplantation but has been used as an adjunct in lung transplant recipients with acute and chronic renal failure or recipients otherwise intolerant of calcineurin inhibitors (131). Concomitant belatacept therapy has allowed for decreased dosing of calcineurin inhibitors and stabilization or improvement in renal function (132), and has been shown to maintain sufficient immunosuppression after withdrawal of CNIs (131).

Conclusions

The challenges of lung transplantation are unique, however the advances made in renal transplantation, bone marrow transplantation and transplant immunology research provide promising insights that are likely to revolutionize the way we manage lung transplantation in the future. In the meantime, ongoing investigations of available medications may help elucidate how to best prevent and treat acute and chronic allograft rejection while minimizing the risk of infection, malignancy and other toxicities of immunosuppression.

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Footnote

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References

1. Linden PK. History of solid organ transplantation and organ donation. Crit Care Clin 2009;25:165-84, ix.
2. Starzl TE, Marchioro TL, Waddell WR. The Reversal of Rejection in Human Renal Homografts with Subsequent Development of Homograft Tolerance. Surg Gynecol Obstet 1963;117:385-95.
3. Murray JE, Merrill JP, Harrison JH, et al. Prolonged survival of human-kidney homografts by immunosuppressive drug therapy. N Engl J Med 1963;268:1315-23.
4. Blumenstock DA, Lewis C. The first transplantation of the lung in a human revisited. Ann Thorac Surg 1993;56:1423-4; discussion 1424-5.
5. Reitz BA, Wallwork JL, Hunt SA, et al. Heart-lung transplantation: successful therapy for patients with pulmonary vascular disease. N Engl J Med 1982;306:557-64.
6. Cooper JD, Pearson FG, Patterson GA, et al. Technique of successful lung transplantation in humans. J Thorac Cardiovasc Surg 1987;93:173-81.
7. Hosenpud JD, Bennett LE, Keck BM, et al. The Registry of the International Society for Heart and Lung Transplantation: sixteenth official report--1999. J Heart Lung Transplant 1999;18:611-26.
8. Trulock EP. Management of lung transplant rejection. Chest 1993;103:1566-76.
9. Karam S, Wali RK. Current State of Immunosuppression: Past, Present, and Future. Crit Rev Eukaryot Gene Expr 2015;25:113-34.
10. Palmer SM, Miralles AP, Lawrence CM, et al. Rabbit antithymocyte globulin decreases acute rejection after lung transplantation: results of a randomized, prospective study. Chest 1999;116:127-33.
11. Brock MV, Borja MC, Ferber L, et al. Induction therapy in lung transplantation: a prospective, controlled clinical trial comparing OKT3, anti-thymocyte globulin, and daclizumab. J Heart Lung Transplant 2001;20:1282-90.

12. Beaufigeau M, Dumesnil D, Carrier M, et al. Seven-year experience with rabbit antithymocyte globulin after cardiac transplantation at the Montreal Heart Institute. Transplant Proc 1997;29:10S-2S.

13. Szczech LA, Berlin JA, Feldman HI. The effect of antilymphocyte induction therapy on renal allograft survival. A meta-analysis of individual patient-level data. Anti-Lymphocyte Antibody Induction Therapy Study Group. Ann Intern Med 1998;128:817-26.

14. Rosenberg PB, Vriesendorp AE, Drazner MH, et al. Induction therapy with basiliximab allows delayed initiation of cyclosporine and preserves renal function after cardiac transplantation. J Heart Lung Transplant 2005;24:1327-31.

15. Opelz G, Dohler B. Lymphomas after solid organ transplantation: a collaborative transplant study report. Am J Transplant 2004;4:222-30.

16. Mazimba S, Tallaj JA, George JF, et al. Infection and rejection risk after cardiac transplantation with induction vs. no induction: a multi-institutional study. Clin Transplant 2014;28:946-52.

17. Christie JD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. J Heart Lung Transplant 2012;31:1073-86.

18. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.

19. Chambers DC, Yusen RD, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-fourth Adult Lung And Heart-Lung Transplantation Report-2017; Focus Theme: Allograft ischemic time. J Heart Lung Transplant 2017;36:1047-59.

20. Penninga L, Moller CH, Penninga EI, et al. Antibody induction therapy for lung transplant recipients. Cochrane Database Syst Rev 2013:CD008927.

21. Furuya Y, Jayarajan SN, Taghavi S, et al. The Impact of Alemtuzumab and Basiliximab Induction on Patient Survival and Time to Bronchiolitis Obliterans Syndrome in Double Lung Transplantation Recipients. Am J Transplant 2016;16:2334-41.

22. Knoop C, Haverich A, Fischer S. Immunosuppressive therapy after human lung transplantation. Eur Respir J 2004;23:159-71.

23. Schnetzler B, Leger P, Volp A, et al. A prospective randomized controlled study on the efficacy and tolerance of two antilymphocytic globulins in the prevention of rejection in first-heart transplant recipients. Transpl Int 2002;15:317-25.

24. Regan JF, Campbell K, Van Smith L, et al. Sensitization following Thymoglobulin and Atgam rejection therapy as determined with a rapid enzyme-linked immunosorbent assay. US Thymoglobulin Multi-Center Study Group. Transpl Immunol 1999;7:115-21.

25. McManigle W, Pavlisko EN, Martinu T. Acute cellular and antibody-mediated allograft rejection. Semin Respir Crit Care Med 2013;34:320-35.

26. Sweet SC. Induction therapy in lung transplantation. Transpl Int 2013;26:696-703.

27. Jaksch P, Ankersmit J, Scheed A, et al. Alemtuzumab in lung transplantation: an open-label, randomized, prospective single center study. Am J Transplant 2014;14:1839-45.

28. Shyu S, Dew MA, Pilewski JM, et al. Five-year outcomes with alemtuzumab induction after lung transplantation. J Heart Lung Transplant 2011;30:743-54.

29. Magliocca JF, Knechtle SJ. The evolving role of alemtuzumab (Campath-1H) for immunosuppressive therapy in organ transplantation. Transpl Int 2006;19:705-14.

30. Whited LK, Latran MJ, Hashmi ZA, et al. Evaluation of Alemtuzumab Versus Basiliximab Induction: A Retrospective Cohort Study in Lung Transplant Recipients. Transplantation 2015;99:2190-5.

31. Swarup R, Allenspach LL, Nemeh HW, et al. Timing of basiliximab induction and development of acute rejection in lung transplant patients. J Heart Lung Transplant 2011;30:1228-35.

32. Grundy N, Simmonds J, Dawkins H, et al. Pre-implantation basiliximab reduces incidence of early acute rejection in pediatric heart transplantation. J Heart Lung Transplant 2009;28:1279-84.

33. Hartwig MG, Snyder LD, Appel JZ 3rd, et al. Rabbit anti-thymocyte globulin induction therapy does not prolong survival after lung transplantation. J Heart Lung Transplant 2008;27:547-53.

34. Garrity ER, Jr., Villanueva J, Bhorade SM, et al. Low rate of acute lung allograft rejection after the use of daclizumab, an interleukin 2 receptor antibody. Transplantation 2001;71:773-7.

35. Borro JM, De la Torre M, Miguelez C, et al. Comparative study of basiliximab treatment in lung transplantation.

Transplant Proc 2005;37:3996-8.

36. van Loenhout KC, Groves SC, Galazka M, et al. Early outcomes using alemtuzumab induction in lung transplantation. Interact Cardiovasc Thorac Surg 2010;10:190-4.

37. Whitson BA, Lehman A, Wehr A, et al. To induce or not to induce: a 21st century evaluation of lung transplant immunosuppression's effect on survival. Clin Transplant 2014;28:450-61.

38. McCurry KR, Iacono A, Zeevi A, et al. Early outcomes in human lung transplantation with Thymoglobulin or Campath-1H for recipient pretreatment followed by posttransplant tacrolimus near-monotherapy. J Thorac Cardiovasc Surg 2005;130:528-37.

39. Scheffert JL, Raza K. Immunosuppression in lung transplantation. J Thorac Dis 2014;6:1039-53.

40. Hopkins PM, McNeil K. Evidence for immunosuppression in lung transplantation. Curr Opin Organ Transplant 2008;13:477-83.

41. Floreth T, Bhorade SM. Current trends in immunosuppression for lung transplantation. Semin Respir Crit Care Med 2010;31:172-8.

42. Valapour M, Skeans MA, Smith JM, et al. OPTN/SRTR 2015 Annual Data Report: Lung. Am J Transplant 2017;17 Suppl 1:357-424.

43. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant 2015;34:1264-77.

44. Parada MT, Alba A, Sepulveda C. Early and late infections in lung transplantation patients. Transplant Proc 2010;42:333-5.

45. Arcasoy SM, Wilt J. Medical complications after lung transplantation. Semin Respir Crit Care Med 2006;27:508-20.

46. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med 2005;353:1711-23.

47. Boumpas DT, Chrousos GP, Wilder RL, et al. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. Ann Intern Med 1993;119:1198-208.

48. Hoes JN, Jacobs JW, Verstappen SM, et al. Adverse events of low- to medium-dose oral glucocorticoids in inflammatory diseases: a meta-analysis. Ann Rheum Dis 2009;68:1833-8.

49. Curtis JR, Westfall AO, Allison J, et al. Population-based assessment of adverse events associated with long-term glucocorticoid use. Arthritis Rheum 2006;55:420-6.

50. W J Bijlsma J, Buttgereit F. Adverse events of glucocorticoids during treatment of rheumatoid arthritis: lessons from cohort and registry studies. Rheumatology (Oxford) 2016;55:ii3-ii5.

51. Shitrit D, Bendayan D, Sulkes J, et al. Successful steroid withdrawal in lung transplant recipients: result of a pilot study. Respir Med 2005;99:596-601.

52. Borro JM, Sole A, De la Torre M, et al. Steroid withdrawal in lung transplant recipients. Transplant Proc 2005;37:3991-3.

53. Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. Immunol Today 1992;13:136-42.

54. Wiederrecht G, Lam E, Hung S, et al. The mechanism of action of FK-506 and cyclosporin A. Ann N Y Acad Sci 1993;696:9-19.

55. Mahalati K, Belitsky P, West K, et al. Approaching the therapeutic window for cyclosporine in kidney transplantation: a prospective study. J Am Soc Nephrol 2001;12:828-33.

56. Grevel J, Welsh MS, Kahan BD. Cyclosporine monitoring in renal transplantation: area under the curve monitoring is superior to trough-level monitoring. Ther Drug Monit 1989;11:246-8.

57. Morton JM, Aboyoun CL, Malouf MA, et al. Enhanced clinical utility of de novo cyclosporine C2 monitoring after lung transplantation. J Heart Lung Transplant 2004;23:1035-9.

58. Trull A, Steel L, Sharples L, et al. Randomized, trough blood cyclosporine concentration-controlled trial to compare the pharmacodynamics of Sandimmune and Neoral in de novo lung transplant recipients. Ther Drug Monit 1999;21:17-26.

59. Flechner SM, Kobashigawa J, Klintmalm G. Calcineurin inhibitor-sparing regimens in solid organ transplantation: focus on improving renal function and nephrotoxicity. Clin Transplant 2008;22:1-15.

60. Gijtenbeek JM, van den Bent MJ, Vecht CJ. Cyclosporine neurotoxicity: a review. J Neurol 1999;246:339-46.

61. Fan Y, Xiao YB, Weng YG. Tacrolimus versus cyclosporine for adult lung transplant recipients: a meta-analysis. Transplant Proc 2009;41:1821-4.

62. Heisel O, Heisel R, Balshaw R, et al. New onset diabetes mellitus in patients receiving calcineurin inhibitors: a systematic review and meta-analysis. Am J Transplant 2004;4:583-95.

63. Moore R, Hernandez D, Valantine H. Calcineurin inhibitors and post-transplant hyperlipidaemias. Drug Saf 2001;24:755-66.

64. Muduma G, Saunders R, Odeyemi I, et al. Systematic Review and Meta-Analysis of Tacrolimus versus Ciclosporin as Primary Immunosuppression After Liver Transplant. PLoS One 2016;11:e0160421.

65. Penninga L, Moller CH, Gustafsson F, et al. Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation: systematic review with meta-analyses and trial sequential analyses of randomised trials. Eur J Clin Pharmacol 2010;66:1177-87.

66. Fung JJ. Tacrolimus and transplantation: a decade in review. Transplantation 2004;77:S41-3.

67. Plosker GL, Foster RH. Tacrolimus: a further update of its pharmacology and therapeutic use in the management of organ transplantation. Drugs 2000;59:323-89.

68. Knoop C, Thiry P, Saint-Marcoux F, et al. Tacrolimus pharmacokinetics and dose monitoring after lung transplantation for cystic fibrosis and other conditions. Am J Transplant 2005;5:1477-82.

69. Ragette R, Kamler M, Weinreich G, et al. Tacrolimus pharmacokinetics in lung transplantation: new strategies for monitoring. J Heart Lung Transplant 2005;24:1315-9.

70. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. European FK506 Multicentre Liver Study Group. Lancet 1994;344:423-8.

71. Group USMFLS. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994;331:1110-5.

72. Keenan RJ, Konishi H, Kawai A, et al. Clinical trial of tacrolimus versus cyclosporine in lung transplantation. Ann Thorac Surg 1995;60:580-4; discussion 584-5.

73. Zuckermann A, Reichenspurner H, Birsan T, et al. Cyclosporine A versus tacrolimus in combination with mycophenolate mofetil and steroids as primary immunosuppression after lung transplantation: one-year results of a 2-center prospective randomized trial. J Thorac Cardiovasc Surg 2003;125:891-900.

74. Zuckermann A, Reichenspurner H. Long Term Follow up of a prospective randomized trial comparing tacrolimus versus cyclosporine in combination with MMF after Lung Transplantation. The Journal of Heart and Lung Transplantation 2003;22:S76-S7.

75. Hachem RR, Yusen RD, Chakinala MM, et al. A randomized controlled trial of tacrolimus versus cyclosporine after lung transplantation. J Heart Lung Transplant 2007;26:1012-8.

76. Treede H, Glanville AR, Klepetko W, et al. Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome: results of a prospective, randomized international trial in lung transplantation. J Heart Lung Transplant 2012;31:797-804.

77. Treede H, Klepetko W, Reichenspurner H, et al. Tacrolimus versus cyclosporine after lung transplantation: a prospective, open, randomized two-center trial comparing two different immunosuppressive protocols. J Heart Lung Transplant 2001;20:511-7.

78. Madoff SD, Kaye J, Newman JS. Postoperative Foot and Ankle MR Imaging. Magn Reson Imaging Clin N Am 2017;25:195-209.

79. Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 2005;56:23-46.

80. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. Transplantation 1996;61:1029-37.

81. Wiesner R, Rabkin J, Klintmalm G, et al. A randomized double-blind comparative study of mycophenolate mofetil and azathioprine in combination with cyclosporine and corticosteroids in primary liver transplant recipients. Liver Transpl 2001;7:442-50.

82. Kobashigawa J, Miller L, Renlund D, et al. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate Mofetil Investigators. Transplantation 1998;66:507-15.

83. Zuckermann A, Klepetko W, Birsan T, et al. Comparison between mycophenolate mofetil- and azathioprine-based immunosuppressions in clinical lung transplantation. J Heart Lung Transplant 1999;18:432-40.

84. Ross DJ, Waters PF, Levine M, et al. Mycophenolate mofetil versus azathioprine immunosuppressive regimens after lung transplantation: preliminary experience. J Heart Lung Transplant 1998;17:768-74.

85. Palmer SM, Baz MA, Sanders L, et al. Results of a randomized, prospective, multicenter trial of mycophenolate mofetil versus azathioprine in the prevention of acute lung allograft rejection. Transplantation 2001;71:1772-6.

86. McNeil K, Glanville AR, Wahlers T, et al. Comparison of mycophenolate mofetil and azathioprine for prevention of bronchiolitis obliterans syndrome in de novo lung

transplant recipients. Transplantation 2006;81:998-1003.

87. Glanville AR, Aboyoun C, Klepetko W, et al. Three-year results of an investigator-driven multicenter, international, randomized open-label de novo trial to prevent BOS after lung transplantation. J Heart Lung Transplant 2015;34:16-25.

88. Strueber M, Warnecke G, Fuge J, et al. Everolimus Versus Mycophenolate Mofetil De Novo After Lung Transplantation: A Prospective, Randomized, Open-Label Trial. Am J Transplant 2016;16:3171-80.

89. Groetzner J, Kur F, Spelsberg F, et al. Airway anastomosis complications in de novo lung transplantation with sirolimus-based immunosuppression. J Heart Lung Transplant 2004;23:632-8.

90. King-Biggs MB, Dunitz JM, Park SJ, et al. Airway anastomotic dehiscence associated with use of sirolimus immediately after lung transplantation. Transplantation 2003;75:1437-43.

91. Ahya VN, McShane PJ, Baz MA, et al. Increased risk of venous thromboembolism with a sirolimus-based immunosuppression regimen in lung transplantation. J Heart Lung Transplant 2011;30:175-81.

92. McWilliams TJ, Levvey BJ, Russell PA, et al. Interstitial pneumonitis associated with sirolimus: a dilemma for lung transplantation. J Heart Lung Transplant 2003;22:210-3.

93. Gullestad L, Iversen M, Mortensen SA, et al. Everolimus with reduced calcineurin inhibitor in thoracic transplant recipients with renal dysfunction: a multicenter, randomized trial. Transplantation 2010;89:864-72.

94. Shitrit D, Rahamimov R, Gidon S, et al. Use of sirolimus and low-dose calcineurin inhibitor in lung transplant recipients with renal impairment: results of a controlled pilot study. Kidney Int 2005;67:1471-5.

95. Knoll GA, Kokolo MB, Mallick R, et al. Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. BMJ 2014;349:g6679.

96. Bhorade S, Ahya VN, Baz MA, et al. Comparison of sirolimus with azathioprine in a tacrolimus-based immunosuppressive regimen in lung transplantation. Am J Respir Crit Care Med 2011;183:379-87.

97. Snell GI, Valentine VG, Vitulo P, et al. Everolimus versus azathioprine in maintenance lung transplant recipients: an international, randomized, double-blind clinical trial. Am J Transplant 2006;6:169-77.

98. Levine DJ, Glanville AR, Aboyoun C, et al. Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation.

J Heart Lung Transplant 2016;35:397-406.

99. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. N Engl J Med 2012;367:2015-25.

100. Ensor CR, Yousem SA, Marrari M, et al. Proteasome Inhibitor Carfilzomib-Based Therapy for Antibody-Mediated Rejection of the Pulmonary Allograft: Use and Short-Term Findings. Am J Transplant 2017;17:1380-8.

101. Safavi S, Robinson DR, Soresi S, et al. De novo donor HLA-specific antibodies predict development of bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant 2014;33:1273-81.

102. Morrell MR, Pilewski JM, Gries CJ, et al. De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. J Heart Lung Transplant 2014;33:1288-94.

103. Tikkanen JM, Singer LG, Kim SJ, et al. De Novo DQ Donor-Specific Antibodies Are Associated with Chronic Lung Allograft Dysfunction after Lung Transplantation. Am J Respir Crit Care Med 2016;194:596-606.

104. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. J Heart Lung Transplant 2010;29:973-80.

105. Hachem RR, Reinsmoen NL. What is the definition of a clinically relevant donor HLA-specific antibody (DSA)? Am J Transplant 2015;15:299-300.

106. Smith JD, Ibrahim MW, Newell H, et al. Pre-transplant donor HLA-specific antibodies: characteristics causing detrimental effects on survival after lung transplantation. J Heart Lung Transplant 2014;33:1074-82.

107. Snyder LD, Gray AL, Reynolds JM, et al. Antibody desensitization therapy in highly sensitized lung transplant candidates. Am J Transplant 2014;14:849-56.

108. Tinckam KJ, Keshavjee S, Chaparro C, et al. Survival in sensitized lung transplant recipients with perioperative desensitization. Am J Transplant 2015;15:417-26.

109. Lund LH, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Heart Transplantation Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1158-69.

110. Gelman AE, Li W, Richardson SB, et al. Cutting edge: Acute lung allograft rejection is independent of secondary lymphoid organs. J Immunol 2009;182:3969-73.

111. Li W, Bribriesco AC, Nava RG, et al. Lung transplant

acceptance is facilitated by early events in the graft and is associated with lymphoid neogenesis. Mucosal Immunol 2012;5:544-54.

112. Sayegh MH, Fine NA, Smith JL, et al. Immunologic tolerance to renal allografts after bone marrow transplants from the same donors. Ann Intern Med 1991;114:954-5.

113. Helg C, Chapuis B, Bolle JF, et al. Renal transplantation without immunosuppression in a host with tolerance induced by allogeneic bone marrow transplantation. Transplantation 1994;58:1420-2.

114. Spitzer TR, Sykes M, Tolkoff-Rubin N, et al. Long-term follow-up of recipients of combined human leukocyte antigen-matched bone marrow and kidney transplantation for multiple myeloma with end-stage renal disease. Transplantation 2011;91:672-6.

115. Fudaba Y, Spitzer TR, Shaffer J, et al. Myeloma responses and tolerance following combined kidney and nonmyeloablative marrow transplantation: in vivo and in vitro analyses. Am J Transplant 2006;6:2121-33.

116. Scandling JD, Busque S, Shizuru JA, et al. Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation. Am J Transplant 2015;15:695-704.

117. Scandling JD, Busque S, Shizuru JA, et al. Induced immune tolerance for kidney transplantation. N Engl J Med 2011;365:1359-60.

118. Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and chimerism after renal and hematopoietic-cell transplantation. N Engl J Med 2008;358:362-8.

119. Kawai T, Cosimi AB, Spitzer TR, et al. HLA-mismatched renal transplantation without maintenance immunosuppression. N Engl J Med 2008;358:353-61.

120. Yamada Y, Boskovic S, Aoyama A, et al. Overcoming memory T-cell responses for induction of delayed tolerance in nonhuman primates. Am J Transplant 2012;12:330-40.

121. Koyama I, Nadazdin O, Boskovic S, et al. Depletion of CD8 memory T cells for induction of tolerance of a previously transplanted kidney allograft. Am J Transplant 2007;7:1055-61.

122. Szabolcs P, Buckley RH, Davis RD, et al. Tolerance and immunity after sequential lung and bone marrow transplantation from an unrelated cadaveric donor. J Allergy Clin Immunol 2015;135:567-70.

123. Madariaga ML, Michel SG, Tasaki M, et al. Induction of cardiac allograft tolerance across a full MHC barrier in miniature swine by donor kidney cotransplantation. Am J Transplant 2013;13:2558-66.

124. Mezrich JD, Benjamin LC, Sachs JA, et al. Role of the thymus and kidney graft in the maintenance of tolerance to heart grafts in miniature swine. Transplantation 2005;79:1663-73.

125. Liu XQ, Hu ZQ, Pei YF, et al. Clinical operational tolerance in liver transplantation: state-of-the-art perspective and future prospects. Hepatobiliary Pancreat Dis Int 2013;12:12-33.

126. Brouard S, Pallier A, Renaudin K, et al. The natural history of clinical operational tolerance after kidney transplantation through twenty-seven cases. Am J Transplant 2012;12:3296-307.

127. Ramos HC, Reyes J, Abu-Elmagd K, et al. Weaning of immunosuppression in long-term liver transplant recipients. Transplantation 1995;59:212-7.

128. Neumann J, Tarrasconi H, Bortolotto A, et al. Acute humoral rejection in a lung recipient: reversion with bortezomib. Transplantation 2010;89:125-6.

129. Stuckey LJ, Kamoun M, Chan KM. Lung transplantation across donor-specific anti-human leukocyte antigen antibodies: utility of bortezomib therapy in early graft dysfunction. Ann Pharmacother 2012;46:e2.

130. Baum C, Reichenspurner H, Deuse T. Bortezomib rescue therapy in a patient with recurrent antibody-mediated rejection after lung transplantation. J Heart Lung Transplant 2013;32:1270-1.

131. Iasella CJ, Winstead RJ, Moore CA, et al. Maintenance Belatacept-Based Immunosuppression in Lung Transplantation Recipients Who Failed Calcineurin Inhibitors. Transplantation 2018;102:171-7.

132. Timofte I, Terrin M, Barr E, et al. Belatacept for renal rescue in lung transplant patients. Transpl Int 2016;29:453-63.

Acute rejection

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Abstract: Despite induction immunosuppression and the use of aggressive maintenance immunosuppressive regimens, acute allograft rejection following lung transplantation is still a problem with important diagnostic and therapeutic challenges. As well as causing early graft loss and mortality, acute rejection also initiates the chronic alloimmune responses and airway-centred inflammation that predispose to bronchiolitis obliterans syndrome (BOS), also known as chronic lung allograft dysfunction (CLAD), which is a major source of morbidity and mortality after lung transplantation. Cellular responses to human leukocyte antigens (HLAs) on the allograft have traditionally been considered the main mechanism of acute rejection, but the influence of humoral immunity is increasingly recognised. As with other several other solid organ transplants, antibody-mediated rejection (AMR) is now a well-accepted and distinct clinical entity in lung transplantation. While acute cellular rejection (ACR) has defined histopathological criteria, transbronchial biopsy is less useful in AMR and its diagnosis is complicated by challenges in the measurement of antibodies directed against donor HLA, and a determination of their significance. Increasing awareness of the importance of non-HLA antigens further clouds this issue. Here, we review the pathophysiology, diagnosis, clinical presentation and treatment of ACR and AMR in lung transplantation, and discuss future potential biomarkers of both processes that may forward our understanding of these conditions.

Keywords: Lung transplant; cellular rejection; acute antibody rejection; humoral rejection

Introduction

Acute rejection of the pulmonary allograft remains a potential cause of acute graft failure but more commonly presents with sub-acute and often subtle graft dysfunction. While arguments continue regarding the risk-benefit of a scheduled surveillance lung biopsy program and indications for therapeutic intervention it is timely to focus on techniques to improve the operating characteristics of surveillance and clinically mandated procedures as well as the kappa scores of the reporting histopathologists. The brave beating of a distant drum heralds the potential utility of gene transcript analysis technology to supplant our

current "gold standard" but while theoretically appealing has not been validated in the crucible of real time clinical practice. Until such time as it is, we are wise to continue quality control measures which improve the safety of the procedure and the adequacy of samples provided to the histopathologists with expert clinical input into final diagnoses and therapies according to our consensus guideline statements.

Acute cellular rejection (ACR)

ACR of the pulmonary allograft is a serious complication

in lung transplantation not only because of potential acute graft dysfunction or failure, but because it is a major established risk factor for the development of chronic lung allograft dysfunction (CLAD) particularly the bronchiolitis obliterans syndrome (BOS). Early detection and treatment of acute rejection is thus of critical importance for lung transplant recipients. Antibody mediated rejection (AMR) may occur concurrently or separately from ACR and requires additional diagnostic and therapeutic manoeuvres and is covered later in the manuscript.

Epidemiology and risk factors

Registry data from the International Society for Heart and Lung Transplantation (ISHLT) show that about a third of patients will have at least one episode of treated rejection in the first year after transplantation (1). Furthermore, acute rejection is responsible for 3.6% of deaths among adult lung transplant recipients in the first 30 days, and 1.8% in the period from 1 to 12 months post-transplant (1). ACR is the most common form of acute lung transplant rejection and is most likely to be diagnosed within the first 6 months following lung transplantation (2).

Compared to the large body of data on the risk factors for CLAD, recipient, immunological and environmental factors predicting ACR are less well studied. Reported risk factors for ACR include the degree of human leukocyte antigens (HLA) mismatching (with discrepancies at certain loci—such as at HLA-DR, HLA-B and HLA-A of greater significance (3), and genetically-determined differences in innate and adaptive recipient immunological responses to the allograft (4,5). Younger age is also associated with a higher rate of rejection in the first year after transplantation, but whether this association is confounded by the indication for transplantation and the underlying disease process is unclear (1). The impact of both induction and maintenance immunosuppression on the incidence of ACR has been studied. Evidence from ISHLT registry data suggests a protective effect of the interleukin-2 receptor antagonists (basiliximab and daclizumab) when compared to no induction, or the use of the CD52 antagonist, alemtuzumab (1). However, there is a paucity of randomised trial data to support this observation, and a systematic review could not find a difference between induction agents (6). With maintenance immunosuppression, tacrolimus is associated with lower rates of BOS than cyclosporine, but the evidence supporting its use over cyclosporine for ACR is less conclusive. Despite Registry data and a single randomized

trial (7) supporting its use, a systematic review could not conclusively demonstrate a benefit (8).

Lastly, infections, particularly community-acquired viral infections like respiratory syncytial virus (RSV), coronavirus, rhinovirus and the influenza and parainfluenza viruses, potentially via stimulation of innate immunity, and exposure of cryptic antigens by epithelial injury, may stimulate alloimmune responses and precipitate ACR.

Mechanisms of ACR

ACR is a response driven by T lymphocytes that recognises foreign major histocompatibility complex (MHC) antigens, also called HLA on allogeneic tissue. The function of MHC is to bind foreign antigenic peptides and display them on the surface of antigen presenting cells (APCs) for presentation to T cells. MHC genes are coded on the short arm of chromosome 6, and are highly polymorphic, resulting in extreme diversity between individuals. This difference in MHC between individuals is the major immunological barrier to transplantation, as the MHC molecules themselves act as antigens and permit rapid recognition of self from non-self.

In cellular rejection of the allograft, two distinct pathways of allorecognition are implicated. In the direct pathway, donor dendritic cells migrate from the graft to the secondary lymphoid tissue ("passenger leukocytes") to present MHC directly to recipient T cells (9). In the indirect pathway, recipient dendritic cells process and present alloantigens derived from dying donor APCs to T cells, either in the secondary lymphoid organs or in the allograft itself (*Figure 1*). Other non-immunological processes, like ischaemia and reperfusion injury or pulmonary infection may cause non-specific activation of local innate immunity which stimulates acute rejection through mechanisms that have not been completely elucidated.

Once recipient T-cells are activated, they undergo clonal expansion and differentiate into alloreactive killer T cells (also called cytotoxic T lymphocytes) which migrate to the graft and dock with the allograft's MHC molecule and initiate tissue destruction (10). Effective blockade of T cell activation and proliferation is required to prevent rejection, and the advent of calcineurin inhibitors was a revolutionary advance in this area.

Clinical manifestations

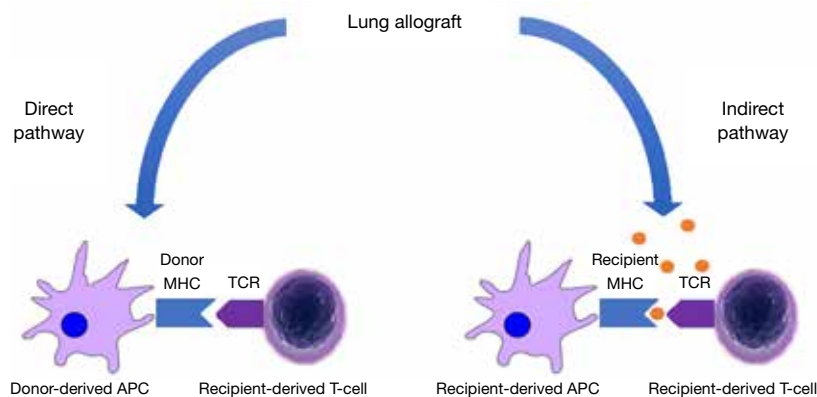Patients with acute rejection present with non-specific

Figure 1 The direct and indirect pathways of allo-sensitisation. In the direct pathway, donor-derived MHC complexes, presented by donor-derived 'passenger' dendritic cells (antigen-presenting cells or APCs), are recognised directly by recipient T cells. In the indirect pathway, host APCs take up immunogenic proteins from apoptotic graft cells and present donor-derived processed peptides on host MHC to host T cells. MHC, major histocompatibility complex; APC, antigen presenting cell.

features that may mimic the many other infectious and post-infectious complications affecting lung transplant recipients. Shortness of breath, cough with or without sputum production and even low-grade fever can be observed in ACR, and there are no signs or symptoms that can reliably be used to distinguish from opportunistic pulmonary infection. Increasing grade of rejection is associated with more severe symptoms (11). Conversely, many episodes of ACR may be diagnosed on routine surveillance trans-bronchial biopsies in the early post-transplant period in asymptomatic patients. The physical examination may be normal, or reveal squeaks or crackles or features of a pleural effusion.

Laboratory findings

There are no specific laboratory findings in ACR. Increasing peripheral blood eosinophil count can precede clinically significant ACR in both lung and heart transplants, but the diagnostic accuracy and positive predictive value of this finding is modest at best (12). A recent review of peripheral blood findings in ACR has suggested that the differential count can be used to guide diagnosis, with an absolute eosinophil count greater than 0.4×10^9 cells, absolute lymphocyte greater than 1.5×10^9 cells, and a relative basophil count greater than 2% favouring rejection, and a neutrophil count greater than 9×10^9 favouring infection (13); however, the diagnostic accuracy of such an algorithm requires formal evaluation in clinical practice.

Pulmonary function tests

Spirometry is an easily performed, non-invasive, safe and repeatable test, routinely performed at follow-up visits or even by patients themselves at home, to screen recipients at risk for rejection. The usual spirometric abnormality in ACR is airflow limitation with reduced forced expiratory volume in one second (FEV_1). A decline in FEV_1 of 10% from a stable baseline that persists for more than 48 hours should generally trigger the need for further investigations (14,15). The utility of spirometry may be less helpful in single lung transplants, where changes may be confounded by progression of disease in the native lung (16). However, stable spirometry does not exclude ACR, and the specificity of spirometry is also low (17). Other airway-centric processes such as acute infection and airway anastomotic problems can cause a similar reduction in FEV_1.

Pulmonary imaging

A chest X-ray is routinely obtained in the investigation of a lung transplant recipient with new respiratory symptoms. In the early post-transplant period, recipients with ACR may demonstrate bilateral pulmonary infiltrates on plain radiography (18), but the sensitivity and discriminant value of a plain radiograph is poor (19). High-resolution computed tomography (HRCT) findings are generally also non-specific (20); however, a recent study found that the presence of bilateral ground-glass opacities with a lower lobe predominance, in association with interlobular septal

thickening, and in the absence of features of fluid overload (cardiomegaly), consolidation and atelectasis, had a positive predictive value of ~90% for acute rejection (21).

Bronchoscopy and transbronchial biopsy

The importance of a histological gold standard for the diagnosis of ACR is clear from the non-specific nature and poor diagnostic accuracy of the clinical parameters or non-invasive investigations listed above. A presumptive diagnosis of ACR requires intensification of immunosuppression, which could potentially be hazardous in the presence of an alternative diagnosis, particularly undiagnosed opportunistic infection. The need to make a confident tissue diagnosis of ACR and to reliably exclude intrapulmonary infection cannot thus be overemphasised. Both may occur simultaneously. Bronchoscopy allows for invasive sampling for histopathological determination, as well as the collection of bronchoalveolar lavage fluid and tissue for cytological and microbiological analysis. The usual practice is to only biopsy one lung in heart-lung or bilateral lung recipients. In cases of radiographic patchy disease, transbronchial biopsies can be directed to the involved areas (22). In the absence of radiological abnormalities or with diffuse disease, trans-bronchial biopsies are routinely obtained from the lower lobe (and, in many centres, also the ipsilateral right middle lobe or lingula, depending on the side biopsied whereas upper lobes are less commonly biopsied); the geographic distribution of rejection grades amongst the lung lobes has been shown to be similar (22), and avoidance of upper lobe biopsies reduces the risk of iatrogenic pneumothorax. The Lung Rejection Study Group (LRSG) recommends a minimum of 5 pieces of evaluable, well-expanded alveolar parenchyma to provide adequate sensitivity for diagnosing ACR (23): a study has shown that this amount can reliably be obtained by performing 10 to 12 biopsy specimens, with a resultant low complication rate (2). Complication rates in lung transplant recipients are low and include, in order of frequency, desaturation (10.5%), haemorrhage more than 100 mL (4%), pneumothorax (0.6–2.5%), post-procedural pneumonia (2%), arrhythmia (0.57%), and the need for mechanical ventilation (0.32%) (2,24,25). Mortality from the procedure has not been reported (*Figure 2*).

Pathology

The histopathological hallmark of ACR is the demonstration on trans-bronchial lung biopsies of a mononuclear inflammatory cell infiltrate centred around small vessel and capillaries, and/or small airways (26). The LRSG of the ISHLT has developed a "*working formulation of the standardization of the nomenclature in the diagnosis of lung rejection*", last revised in 2007, which establishes the diagnostic criteria for ACR and objectively grades its severity (*Table 1*) (23). Perivascular inflammation, termed "A-grade", evaluates the presence and extent of mononuclear cell invasion—composed chiefly of T cells, although B-cells and eosinophils have also been described (27)—around the blood vessels, surrounding submucosal interstitium and alveolar walls. The grades range from A0 (no rejection) to A4 (severe). Airway inflammation, termed "B-grade" rejection, evaluates the lymphocytic response in the submucosa of bronchioles, which may extend through the basement membrane at higher grades. The interpretation of B-grade rejection is problematic as airway tissue is often not represented on a trans-bronchial biopsy specimen, and airway-centric mononuclear inflammation is not specific and can also be seen during episodes of acute infection. The finding of significant airway neutrophilia, necrosis, granulomas and viral cytopathic effect together perivascular or peribronchiolar inflammation may favour infection over rejection, although it is important to remember that the two processes are not mutually exclusive and can occur contemporaneously. In general, the LRSG advises grading both A- and B-grade rejection only after the rigorous exclusion of infection (28,29). Other processes that can confound the histological diagnosis of ACR include drug toxicity, aspiration, ischaemia/reperfusion injury, AMR and recurrent primary disease (26).

The sensitivity of trans-bronchial lung biopsy for diagnosing ACR is only ~70%, and is highly dependent on the experience of the histopathologist (30). Despite attempts to standardise the histological interpretation of ACR, inter-observer agreement has been modest. The 2007 revision has attempted to improve this, but follow-up studies have not shown increased concordance (31).

A promising technique being studied to increase the diagnostic yield in ACR by obtaining more lung tissue is cryobiopsy (32). Cryobiopsy is a relatively new interventional bronchoscopic technique that yields larger specimens with better representation of airways, alveoli and vascular structures. In the cryobiopsy procedure, a flexible cryoprobe delivers a compressed gas at the tip of the probe which freezes tissue on the probe's tip. Disadvantages are the need for intubation, and there are theoretical concerns

Grade A1 (minimal)   Grade A2 (mild)

Grade A3 (moderate)   Grade A4 (severe)

Grade B1R (low-grade)   Grade B2R (high-grade)
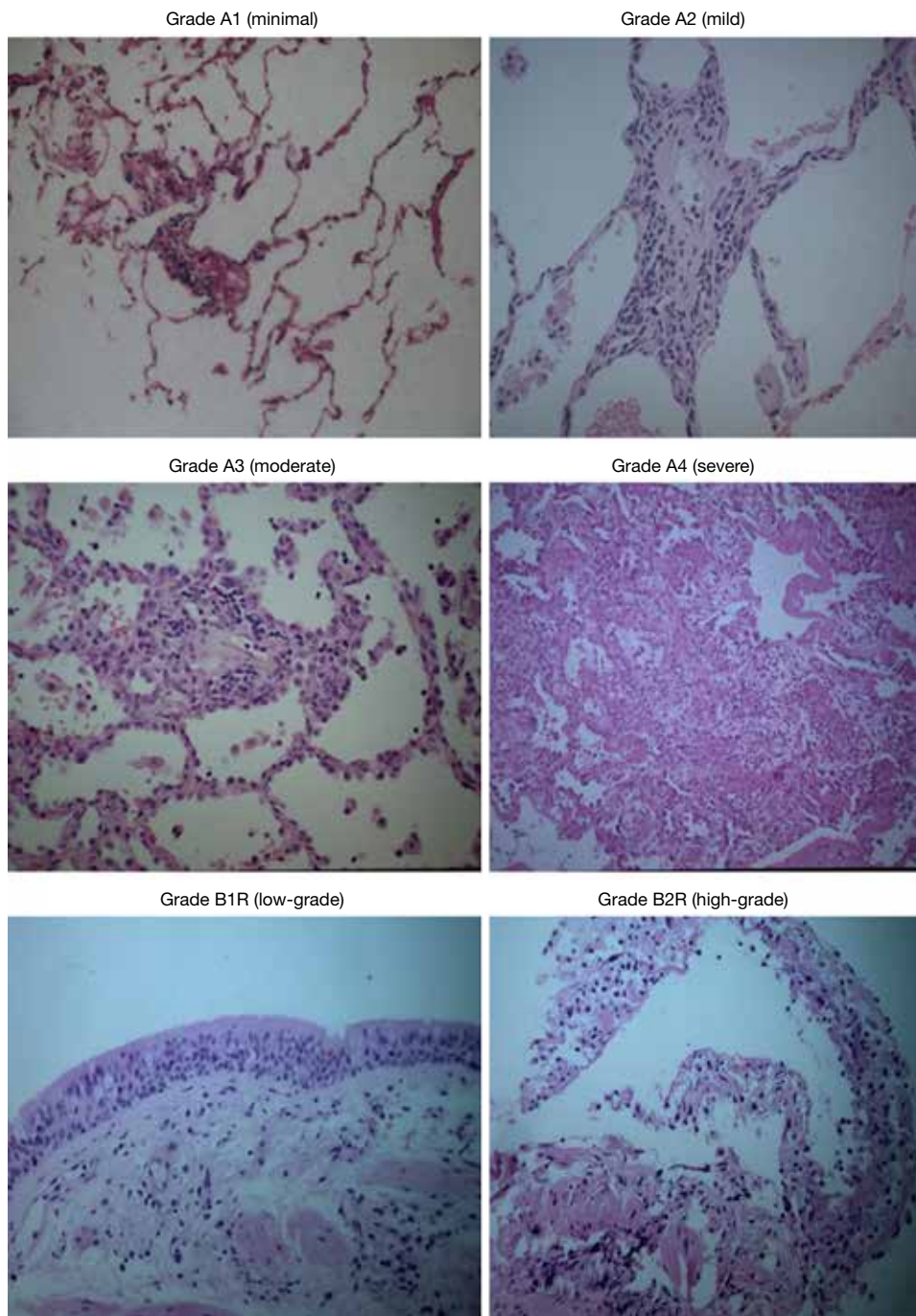
Figure 2 Trans-bronchial biopsies at high-power field demonstrating the different grades of rejection.

about higher risks of pneumothorax and haemorrhage. A recent study of cryobiopsy in lung transplant recipients, however, found more representative specimens, with less crush artefact, and without a higher rate of complications when compared to conventional biopsies (33).

Surveillance bronchoscopy in asymptomatic recipients

The role of surveillance trans-bronchial biopsies in asymptomatic lung transplant recipients, at regular intervals is controversial and is not universally practised

Table 1 Pathological grading of ACR*

Grade	Meaning	Appearance
A-GRADE: perivascular inflammation		
0	None	Normal lung parenchyma
1	Minimal	Scattered, infrequent small mononuclear perivascular infiltrates; no eosinophils
2	Mild	More frequent perivascular infiltrates identifiable at low magnification; eosinophils may be present
3	Moderate	Dense perivascular infiltrates, eosinophils and neutrophils common. Pathognomonic feature is extension into alveolar septae and airspaces
4	Severe	Diffuse perivascular, interstitial and air-space infiltrates with pneumocyte damage and features of acute lung injury
B-GRADE: airway-associated inflammation		
0	None	No evidence of bronchiolar inflammation
1R	Low grade	Single-layer mononuclear cells in bronchiolar submucosa
2R	High grade	Larger infiltrates of larger and activated lymphocytes in bronchiolar submucosa, with potential involvement of eosinophils and plasmacytoid cells
X	Ungradable	No bronchiolar tissue available

*, adapted from the 2007 Working Formulation of the ISHLT (23); "R" in B-grade inflammation denotes "revised". ACR, acute cellular rejection.

amongst lung transplant centres. Schedules for surveillance bronchoscopies vary, but generally involve monthly biopsies in the immediate post-transplant period (when the incidence of ACR is the highest), and continue at less regular intervals for the period from 3 to 12 months after transplant. The rationale for surveillance is the high incidence of asymptomatic ACR (~25%) observed (25), and the suboptimal diagnostic accuracy of non-invasive tests (described above). Prospective cohort studies have shown that the incidence of high-grade perivascular or airway inflammation on routine surveillance biopsies is 16% and 14%, respectively (25,34). However, despite this finding, the evidence that early detection of ACR with subsequent augmentation of immunosuppression alters outcomes in lung transplantation is lacking. A small study randomising patients to either surveillance bronchoscopy or clinically-indicated biopsy did not find a difference in acute rejection, the development of CLAD or survival (35).

Other biomarkers

The identification of an objective, repeatable and specific non-invasive test that could replace trans-bronchial biopsy—which, as has been discussed, is an imperfect gold standard with modest inter-observer reliability—

for the diagnosis of ACR remains an attractive but elusive goal. Furthermore, in the absence of a suitable biomarker, the current practice has also been to use the serum drug levels of calcineurin inhibitor as a proxy for the degree of immunosuppression, the intensity of which has been shown to correlate with a lower incidence of acute rejection (11,36). This latter approach has obvious drawbacks, not least the pharmacokinetic and pharmacogenetic variation between individuals, and the future identification of a biomarker more indicative of the underlying state of immune activation and/or rejection would be a much better guide to inform ongoing immunosuppression prescription. Several potential biomarkers are currently being evaluated. Exhaled nitric oxide (FeNO) as a marker of underlying lung inflammation in lung transplant recipients has been well studied, and levels are significantly elevated compared to baseline values in patients with ACR (37) (especially in those with lymphocytic airway inflammation), but the specificity is poor, as pulmonary infections also increase FeNO (38,39).

Quantification of donor-derived cell free DNA (ddcfDNA) from the allograft in circulating plasma (detectable by next-generation sequencing) is an exciting blood-based biomarker in solid organ transplantation. Preliminary work suggests that increased levels of ddcfDNA are associated with both acute and chronic rejection,

correlate closely with histopathological features of ACR, and are associated with declines in lung function (40). However, validation studies are still required and optimal cut-off values are yet to be determined.

There are also several direct functional tests of immune function, some of which are commercially available. The Cylex Immune Cell Function Assay (ImmuKnow; Cylex, Inc., Columbia, MD, USA) measures ATP synthesis in activated CD4 T cells after exposure to a stimulant (phytohemagglutinin) as a measure of cell-mediated immunity, but its sensitivity for ACR seems to be low (41). The alloreactive T-cell frequency assay can demonstrate increased CD4, CD8 and Treg proliferation by mixed lymphocyte reaction to donor antigens in ACR, but its clinical application is limited by the complexity of the assay and other alloimmune responses (42).

Lastly, transcriptome signatures of ACR can be studied. MicroRNAs (miRNA) are small non-coding RNA molecules that are critical controllers of post-transcriptional regulation of gene expression. Several studies have implicated their involvement in renal and heart transplant rejection (43,44), and a small study in lung transplant recipients identified a distinct miRNA signature in airway epithelium that distinguished patients with ACR from those without rejection (45). Even more promising is that miRNA profiles that detect cardiac ACR have been found in peripheral blood, and the potential of miRNA as a blood-based marker of ACR is an exciting future field of study.

Treatment of ACR

Treatment decisions are dependent on institutional practice and the histological grade of rejection. Whilst there is consensus about treating higher grades of acute perivascular rejection (symptomatic grade A2 and above), the management of minimal or mild rejection (A1, asymptomatic grade A2, or isolated B-grade rejection) is more controversial. Some centres will elect not treat in the latter situations, rather repeating the biopsies in 4–6 weeks' time. However, considering that minimal rejection has been shown to be associated with an increased risk of CLAD comparable to higher grades of rejection (46), and that lymphocytic bronchiolitis (B-grade rejection) has also been shown to be independently associated with increased risk of CLAD and death (34), both types and all grades of ACR should probably be strongly considered for treatment, especially in patients with a clinical syndrome suggestive of rejection. A special consideration may be the asymptomatic patient with minimal A1 rejection (and no

B-grade rejection) detected on surveillance biopsies in whom augmentation of immunosuppression may considerably jeopardise the treatment of a current and serious opportunistic viral or fungal infection—in this situation, deferment of treatment and re-biopsy may be a safer strategy.

The cornerstone of treatment for ACR is the steroid pulse. However, there are no study data to guide the dose or duration of therapy. Most centres advocate pulsed-dose methylprednisolone for ~3 days with transition to a tapering oral steroid wean. Some clinicians will use only oral prednisolone (0.5–1.0 mg/kg) for milder grades of rejection, although evidence for this practice is lacking. Most centres which perform surveillance bronchoscopies will also perform a follow-up bronchoscopy 4–6 weeks after an episode of ACR. This practice is supported by the finding of significant rates of persistent ACR that are often clinically occult (47,48).

The management of persistent or refractory ACR is not well established. Failure to respond to treatment for ACR should trigger investigations for concomitant AMR. Together with a repeat steroid pulse, other changes to therapy in cases of persistent ACR may include a switch to tacrolimus (if on a cyclosporine-based regimen) (49,50) or the addition of a mammalian target of rapamycin (mTOR) inhibitor, such as everolimus. Other potential treatments that can be considered, if available, include alemtuzumab (an antibody to CD52, a peptide present on the surface of mature lymphocytes, which causes antibody-dependent lysis of lymphocytes) (51), and extracorporeal photophoresis (ECP). ECP is thought to reduce immune responses in transplant recipients by stimulating and expanding the number of peripheral regulatory T-cells, and has been shown to stabilise recurrent ACR and ameliorate lung function decline in CLAD (52).

AMR

We have discussed acute rejection post lung transplantation (LTX) as a cellular driven immune response, which has historically been considered the main mechanism of acute lung allograft rejection. Over the past decade however, the role played by antibodies in acute lung allograft, as well as CLAD has stimulated growing interest (53-56) and AMR has evolved from a hypothetical and controversial concept (23), to an important diagnostic consideration in patients with acute allograft dysfunction and a well-recognised clinical entity post lung transplantation (56).

Much of our initial understanding of pulmonary AMR

has been extrapolated from the renal and heart transplant literature, where AMR has been recognised for some time (57,58). One of the biggest difficulties facing clinicians in reaching a diagnosis of AMR and choosing suitable management strategies is the lack of standardised data available, with different studies using various criteria for its diagnosis. A significant development has been the publication of the first consensus document on pulmonary AMR by the ISHLT. This consensus document has provided much needed standardisation of the criteria used to define AMR, which will facilitate universal interpretation of future research. It is generally accepted that this is an active document and ongoing research as well as increasing numbers of recognised pulmonary AMR diagnoses will generate information to help us understand the pathophysiology of pulmonary AMR and address numerous questions, which as yet remain unanswered (56).

In this section we will address the pathophysiology, diagnosis, clinical presentation, classification, and treatment of AMR, and explore some of the diagnostic and management challenges.

Pathophysiology of AMR

AMR results from the recipient's immunological system's ability to recognize donor HLA present in the lung allograft thereby inducing an allogeneic immune response resulting in the production of donor-specific antibodies (DSA) by B cells and plasma cells. These DSA target donor HLA expressed on donor organ capillary endothelial cells. HLA antigens exist as two distinct classes. The HLA class I antigens (HLA-A, HLA-B, HLA-C) are expressed in both T Cells and B cells, whereas HLA class II antigens (HLA-DP, HLA DQ, HLA-DR) are only expressed in B cells.

The polymorphic nature of HLA antigens provides up to 2×10^4 different endogenous antigens that can be presented to T Cells with subsequent activation of B cells and plasma cells to produce DSA that target the graft (59). These DSA may be formed prior to transplant in patients who have been previously sensitized following exposure to allogeneic tissue via previous pregnancy, blood transfusions, or transplantation (60,61), or they may also develop *de novo* post LTX. The rate of detection of DSA by single antigen bead (SAB) testing in various studies ranges from 10–61% of patients within 1 year post lung transplant (62-66), and their detection in post-transplant serum is associated with a significantly increased risk of AMR (67-69) and CLAD (62,65,66,68,70-73).

The significance of pre-transplant DSA has been well established in kidney and heart transplants, and in lungs they have been associated with acute and chronic effects on the lung allograft including refractory acute rejection, lymphocytic bronchiolitis (74), AMR (75), and CLAD (72,76,77). Pre-formed DSA have also been reported to promote *de novo* DSA development early after transplant and impact patient survival (64,78,79).

Graft injury as a result of DSA can occur via complement-dependent and complement-independent mechanisms. The initial activating step is the formation of the antigen-antibody complex, which results in an amplified immune response with numerous downstream effects (56). In the complement-dependent pathway, otherwise known as the classical pathway, antibody binds to its corresponding HLA-antigen on the airway epithelium and the resulting antigen-antibody complex activates the complement cascade. However, complement is not always necessary for antibodies to cause graft damage, as they are able to directly act on inflammatory cells such as macrophages, natural killer cells, and neutrophils, via interaction with the Fc receptor portion on the antibody, and induce pro-inflammatory cytokine production and microangiopathy (78,80). Not all patients with DSA present in their serum exhibit other features of AMR. This could be due to the variable pathogenicity of the IgG subclass itself. There are 4 different IgG subclasses—IgG1, IgG2, IgG3, and IgG4, each with variable affinity for binding to Fc receptors. IgG4 and IgG2 are usually considered to be non-complement binding and IgG3 and IgG1 bind complement with the former having the strongest affinity to the Fc receptor. However, when there is high antigen, epitope level, or increased complement concentration, all IgG subclasses have the ability to activate complement (81). This may therefore be a case of a "wolf in sheep's clothing", where DSA that may previously have been considered relatively benign, can become pathogenic depending on changes within immunological environment such as inflammatory responses to certain triggers (82,83).

DSAs to HLAs have traditionally been considered responsible for AMR lung allograft injury. However some patients meet criteria for AMR but have no evidence of HLA-DSA and there is growing evidence to support the role of non-HLA DSA or tissue self-antigens (SAGs) which are directed towards epithelial antigens, such as K-alpha 1 tubulin (Kα1T) and Collagen V (Col-V). These may account for the unmeasurable HLA-DSA in some patients with AMR (84-89). Similarly, there are patients in whom DSA are clearly present yet they do not exhibit other

manifestations of AMR, which suggests alternative factors may influence the susceptibility or risk of rejection in the presence of antibodies that can bind the graft (90).

Significant work has been done by Bharat and his colleagues on SAGs. Their work has demonstrated that up to 30% of patients undergoing lung transplantation have pre-existing antibodies against Col-V and Kα1T, which strongly predispose patients to primary graft dysfunction (PGD), development of *de novo* alloimmunity, and CLAD (86,88). Their group have introduced the concept of the 'two hit' hypothesis for the development SAGs. The initial 'hit' involves an initial lung injury as a result of triggers such as infections with community acquired respiratory viruses (CARV), gastro-oesophageal reflux disease (GORD), ischaemia-reperfusion injury, or even the presence of HLA-DSA (91,92). The resulting local inflammatory response exposes SAGs such as Col-V and Kα1T which stimulate up-regulation of self-reactive lymphocytes and non-HLA antibody production. The second 'hit' is a result of down-regulation of regulatory T cells (TREGS) with loss of inhibition of self-reactive lymphocytes against SAGs which would usually induce T cell tolerance to SAGs. This ultimately leads to SAG autoimmunity Bharat (91,93). These SAGs may be a significant contributing factor to ongoing CLAD in patients who clear DSA post AMR treatment using commercially available kits that do not measure SAGs. A study by Hachem *et al.* has previously shown that more than 96% of LTX recipients with pre-existing HLA-DSA developed *de novo* SAGs within three years of LTX and were strongly predisposed to development of CLAD. However, AMR treatment was only effective in reducing the increased risk of CLAD if SAGs were cleared as well as HLA-DSA. Patients who cleared HLA-DSA but had persistent SAGs shared an equal risk of CLAD as those with both HLA-DSA and SAGs (34). Ongoing inflammation in patients post LTX recipients and those with end-stage lung disease provides an ideal environment for the above hypothesis, particularly in patients with cystic fibrosis (CF) who have ongoing infection and patients with idiopathic pulmonary fibrosis (IPF) who have ongoing inflammation. Studies have demonstrated a higher prevalence of SAGs pre-transplant in patients with CF and IPF compared to patients with other conditions such as COPD, whereas other studies have shown this can lead to the expansion of auto-reactive lymphocytes. Individuals with these diseases have the highest prevalence of SAGs pre-LTX compared to patients with other diseases such as COPD and alpha-1 anti-trypsin deficiency (89,94-96).

Future research will no doubt help clarify the association between the pathophysiology of AMR (67-69) and that of CLAD (62,65,66,69-72). However, more data are needed to prove a link between these phenomena (97) .

Histopathological features of AMR

Trans-bronchial biopsies, when safe to perform, are an essential component of the diagnostic algorithm for patients presenting with a drop in lung function of more than 10% from baseline. It is considered the 'gold standard' for the diagnosis of ACR, however the appearance of 'capillary injury' with neutrophil margination, neutrophil capillaritis, and arteritis are considered by pathologists to be suggestive of AMR (23) and help clinicians make a more confident diagnosis by addressing the diagnostic criteria currently mandated by the ISHLT (56). These morphological features are suggestive of AMR but are not pathognomonic, and can occur in other disease processes involving the lung such as infection, organising pneumonia, and diffuse alveolar damage (DAD) secondary to other causes, as well as ACR (grade > A3) (56,61,98).

As in the case of DSAs, the absence of these features does not exclude AMR, and once again the clinical context needs to be taken into consideration when contemplating the diagnosis, and an effort needs to be made to exclude other causes such as infection although the two may co-exist (56,99) although the two may co-exist.

C4d immunohistochemistry

The identification of C4d staining on a biopsy sample as a surrogate marker for AMR due to activation of the complement cascade is controversial and its role in the diagnosis of pulmonary AMR may have been overestimated (56,61). It is rarely seen in patients with a diagnosis of pulmonary AMR based on other criteria (26,100), and there is poor inter-pathologist agreement when it comes to recognising a positive stain on trans-bronchial biopsy (101). Notably, C4d is not specific to pulmonary AMR, can be found in any process that is associated with complement activation such as reperfusion injury and infection (86), and studies have shown no little or correlation between positive staining and the presence of DSA (100,102).

Clinical features of AMR

The clinical presentation of pulmonary AMR can be

variable depending on the time post-transplant. Hyperacute rejection can occur as soon as the vascular anastomosis is completed and recipient immune cells come into contact with the donor recipient endothelial cells. Pre-formed antibodies already present at the time of transplant are solely responsible for the devastating effects of this type of rejection. Graft failure occurs within minutes to hours and is usually fatal as a result of acute severe refractory hypoxaemia with respiratory failure. Diffuse pulmonary infiltrates are seen on radiological examination, and histopathology reveals neutrophilic margination, vasculitis, fibrinoid necrosis, vascular thrombosis, and pulmonary infarction (61). With the introduction of highly sensitive assays that can detect DSA the incidence of hyperacute rejection has been significantly reduced (103), however antibody responses to donor HLA antigens are not well controlled with current conventional immune-suppression and therefore acute AMR can occur at any time after transplantation (104), with either pre-transplant HLA, *de novo* HLA, or non-HLA DSA such as autoantibodies to Kα1T and Col-V being responsible in mediating allograft injury. A more chronic and indolent form for AMR is thought to contribute to the development of CLAD due to BOS or even r-CLAD, but there are insufficient data to confirm this association (97).

A high index of suspicion is necessary when considering pulmonary AMR as a possible diagnosis as its clinical presentation is often nonspecific. Patients may often present with an asymptomatic drop in lung function and non-specific symptoms such as cough, dyspnoea, fever, lethargy, which can rapidly progress to profound dyspnoea with progressive respiratory failure requiring increasing oxygen supplementation, mechanical ventilation and in some cases ECMO as an attempt to buy time for rescue therapy to work (105).

Diagnosis of AMR

The diagnosis of AMR is challenging and a high level of clinical vigilance is paramount. The current classification system requires evidence of lung allograft dysfunction, histological changes consistent with AMR, positive C4d staining, and the presence of DSA. A multidisciplinary approach that addresses the need to consider the clinical presentation as well as immunological and pathological information obtained during the diagnostic process is therefore recommended (56,98) and includes:

(I)   Measurement of allograft function;

(II)   Histopathological assessment including C4d staining;

(III)   Evaluation for the presence of DSA.

The diagnosis of AMR is usually made during the process of investigating a patient for a drop in lung function of greater than 10% from their baseline. This usually involves radiological investigations including a computerised tomography scan of the chest, nasopharyngeal swabs for respiratory viruses, bronchoscopy and bronchoalveolar lavage to look for infectious aetiologies as well as any airway abnormalities that may be contributing to the loss of graft function. If safe to do so, a trans-bronchial biopsy is also undertaken as the 'gold standard' in diagnosing ACR, and allows assessment for any features consistent with AMR, which includes staining for C4d (54) (*Figure 3*). Serology for the detection of DSA using SAB is also requested.

The identification of DSA has evolved over time from the provision of a rather crude measure of the presence of DSA by mixing donor lymphocytes with recipient serum with the finding of recipient cell lysis indicating the presence of DSA, the so called complement-dependent cytotoxic (CDC) assay (106). A modified version of this test relied on the use of a panel of HLA-phenotyped cells to express the result as the percentage of cells in the panel giving a positive result, the "panel reactive antibody" (PRA) percentage, with higher percentage PRA indicating a higher probability of positive crossmatch and vice versa (107).

The development of the solid phase SAB technology has allowed for very sensitive and accurate identification of DSA. The assay involves incubation of the patient's serum with beads coated with two fluorochromes, which result in a unique signal for each bead. A specific HLA molecule produced by recombinant technology is also attached to each bead. When the recipient's serum is incubated with the beads, any HLA-antibody present will react with the bead expressing the corresponding antigen molecule. The beads are then washed against a fluoro-labelled anti-human IgG antibody, which provides a mean fluorescence intensity (MFI) representing the amount of fluoro-labelled anti-IgG in complex with anti-HLA antibody. The MFI is not an accurate quantitative measurement of the amount of antibody concentration which requires measurement of the antibody titre (108-110).

Given the above, it is perhaps not surprising that there is ongoing controversy regarding what cut off level of MFI should be considered a clinically significant positive result. Whereas titres are more informative and accurate , random arbitrary selection of MFI thresholds to determine

positive SAB results have led to conflicting reports on the clinical significance of DSA and their relationship to clinical outcomes post transplantation, with some studies using low MFI thresholds [100–1,200] showing no negative effect on transplant outcome with pre-existing DSA (62,111), whereas other studies that have chosen a slightly higher MFI (>3,000) showing an association with increased AMR risk following lung transplantation (75). Notably this study reported a lack of effect of DSA with MFI values of 1,000–3,000, clearly demonstrating the importance of selecting MFI threshold values for identifying anti-HLA antibodies. The MFI threshold may explain suggested differences between pre-transplant DSA against Class I and II HLA associated with increased risk for BOS and decreased survival as suggested by the study conducted by Brugiere *et al*. where they showed that DSA against class II HLA, but not class I, was associated with increased risk for BOS and decreased survival, though there was significant difference in the MFI values for anti-class I (1,048±256) and anti-class II (2,175±597) DSA, precluding definitive assessment of differences in biological impacts between antibodies against class I or class II HLA (76).

Longitudinal variability in the MFI levels for particular antibodies are also seen which is thought to be due to the deposition of antibodies in the graft leading to levels in the circulation, the so called 'sponge effect' as described by Girnita and colleagues who noted the detection of DSA in the serum only after removal of the graft suspected to have AMR (73).

It is also unclear as to whether DSA to any HLA locus or DSA with any MFI would have the same clinical outcome (111). Some studies have shown that HLA class II antibodies may be more significant than HLA Class I antibodies (73,112), and that patients who develop class II DSA or have persistent DSA despite antibody-depleting therapy have worse long term outcomes after transplantation (67,113,114).

C1q binding

The C1q binding assay C1q screen™ (One Lambda Inc., Canoga Park, California, USA), was developed in an attempt to determine which patients with detectable DSA are at higher risk of graft injury based on the presence of complement fixing DSA. As much as it is informative it only recognizes binding and not physiological complement activation (115-117). However, its clinical utility has been demonstrated in renal (117), heart (118), and lung

transplants (67).

Complement is not always required to cause antibody mediated graft injury since DSA have the ability to induce damage via non-complement dependant pathways, and this is a significant limitation to using C1q binding to predict the likelihood of an a antibody binding complement resulting in allograft damage (119).

Classification of AMR

AMR is classified into clinical and sub-clinical AMR. Clinical AMR indicates that there is allograft dysfunction whereas in sub-clinical AMR the lung function is normal. Clinical AMR is then sub-classified into definite, probable and possible AMR. There needs to be allograft dysfunction for it to be considered clinical AMR. In addition 4 other criteria are considered which include exclusion of other causes, histological changes consistent with AMR, the presence of C4d staining on biopsy, and the presence of DSA. When all criteria are met it is definite AMR. When 3 out of 4 criteria are met it is classified as probable AMR, whereas the presence of 2 out of 4 criteria classifies it as possible AMR. Subclinical AMR is also sub-classified into definite, probable and possible, however there is no allograft dysfunction, and definite subclinical AMR is when there is histology consistent with AMR, positive C4d staining and presence of DSA. Probable subclinical AMR is consistent with the presence of 2 of 3 criteria and possible AMR 1 of 3 criteria (*Table 2*, *Figure 4*) (56).

Hyperacute rejection can occur peri-operatively, acute rejection as discrete clinically-symptomatic episodes and chronic rejection may manifest as persistent allograft dysfunction resulting from cumulative and on-going pathologic events. Rates of hyperacute rejection have significantly been reduced as a result of the introduction of highly sensitive assays used at the time of cross-matching (103).

Treatment of AMR

The management of AMR poses an ongoing dilemma for physicians caring for patients in whom a diagnosis of AMR has been made since there is no clear evidence to determine the best management option for AMR post lung transplantation. There are no randomised controlled or head to head studies and current data supporting various treatment modalities arise from retrospective studies, case reports, or case series. Current treatment strategies aim to deplete and modulate the

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Table 2 Definition and diagnostic certainty of clinical pulmonary AMR depending on criteria present*

AMR Grade	Allograft dysfunction	Other causes excluded	Lung histology	Lung biopsy C4d	DSA
Definite	X	X	X	X	X
Probable	X	X	X	O	X
Probable	X	X	X	X	O
Probable	X	X	O	X	X
Probable	X	O	X	X	X
Possible	X	X	X	O	O
Possible	X	X	O	O	X
Possible	X	X	O	X	O
Possible	X	O	X	X	O
Possible	X	O	X	O	X
Possible	X	O	O	X	X

*, adapted from the 2016 pulmonary AMR consensus document of the ISHLT. X, absent; O, present. AMR, antibody-mediated rejection; DSA, donor-specific antibodies.
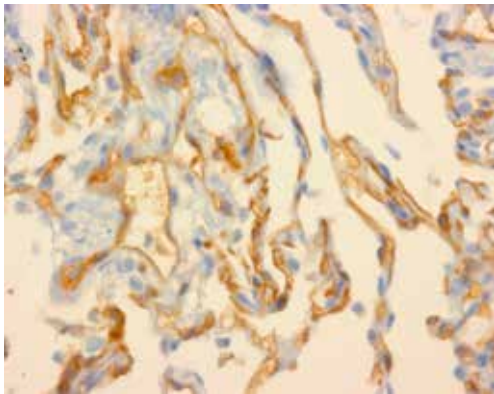

Figure 3 Trans-bronchial biopsy at high power magnification showing C4d capillary staining.

production of circulating DSA in an attempt to arrest graft dysfunction. As yet, there are no clear guidelines or consensus on the management of AMR post lung transplantation. Current treatment regimens are based on those employed in other solid organ transplants and include the use of plasmapheresis (PP), intravenous human immunoglobulin (IVIG), rituximab, and bortezomib (120), but results are suboptimal (121). PP removes antibodies from the circulation and has been reported to ameliorate lung function in some studies. IVIG causes B-cell apoptosis, reduces B-cell numbers, blocks binding of donor reactive antibodies and may inhibit complement activation (122).

Rituximab is a chimeric human/murine monoclonal antibody directed at the CD-20 molecule that is found on mature B cells but not on pro-B cells or plasma cells and therefore it leads to elimination of peripheral B cells in the circulation but it has no effect on mature plasma cells or B cells in lymphoid tissue (123). Bortezomib is a proteasome inhibitor that reduces DSA by depleting plasma cells by causing plasma cell apoptosis. Its clinical use has been supported within the renal (124-128), and lung transplant literature (67,129), and has been effectively used to treat AMR that has been refractory to other treatments. The administration of bortezomib subcutaneously is associated with less side effects such as leucopoenia, neutropenia, thrombocytopenia, and peripheral neuropathy (130). Other monoclonal antibodies which have been used as salvage treatment for AMR include eculizumab and alemtuzumab. Eculizumab is an anti-C5 monoclonal antibody that blocks terminal complement activation. Alemtuzumab is a monoclonal antibody that binds to CD52, a protein present on the surface of mature lymphocytes (131,132).

Once treated, pulmonary AMR may stabilise, progress, or improve. A complete response to treatment is defined as a return to baseline function, abolition of DSA and reversal of pathologic changes. Partial response is improvement in lung function but not all parameters return to baseline. Stabilisation is no further clinical deterioration. No response is defined as on-going clinical deterioration and continued pathology (56).
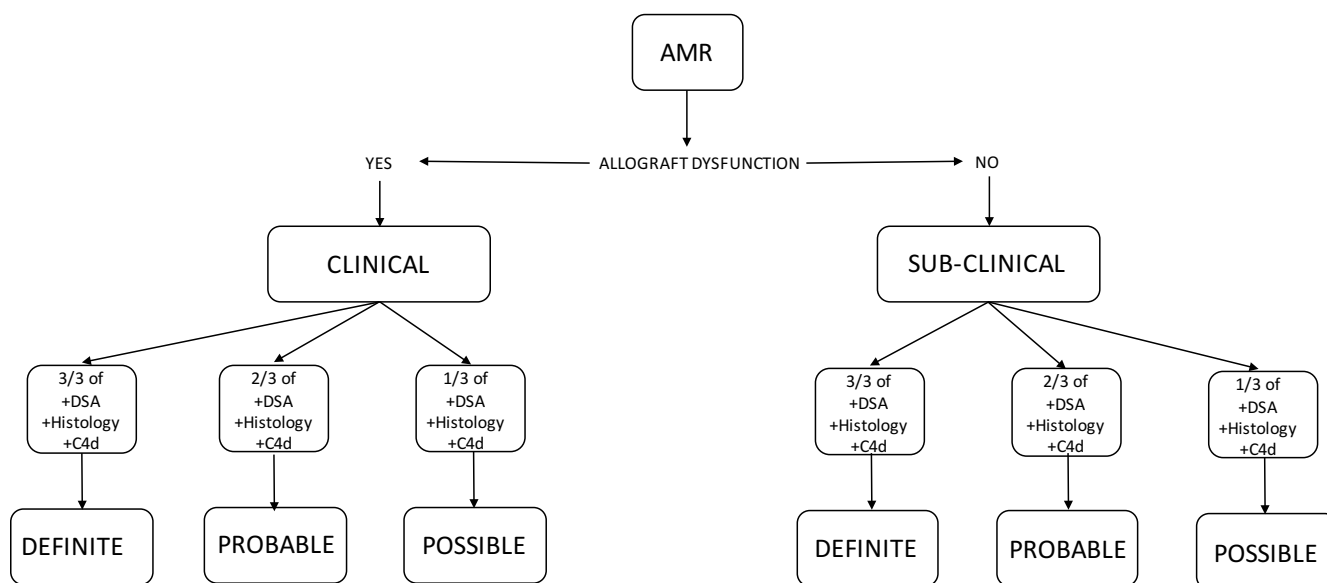
Figure 4 Classification of AMR according to the presence or absence of diagnostic certainty and presence (Clinical) or absence (Sub-clinical) of allograft dysfunction*. *Adapted from the 2016 pulmonary AMR consensus document of the ISHLT. AMR, antibody-mediated rejection.

Conclusions

Lung transplantation is unique to other solid organs in that it is open to the environment and undergoes continuous stimulus from infectious and non-infectious stimuli, which may play a part in up-regulating the immune system and therefore higher immune suppression is necessary. Long-term survival remains disappointing, with CLAD being the main cause of death. Current immunosuppression strategies targeting the T-cell responses do not seem to control CLAD, which may suggest an alternative pathway mechanically causing on-going graft damage. AMR has taken centre stage recently as a possible important mediator of chronic allograft injury and with improved knowledge on its mechanism of injury we may be able to identify targeted treatment that will enable us to gain good control and overall improve our patient survival post transplantation.

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None.

Footnote

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References

1. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.

2. Hopkins PM, Aboyoun CL, Chhajed PN, et al. Prospective analysis of 1,235 transbronchial lung biopsies in lung transplant recipients. J Heart Lung Transplant 2002;21:1062-7.

3. Quantz MA, Bennett LE, Meyer DM, et al. Does human leukocyte antigen matching influence the outcome of lung transplantation? an analysis of 3,549 lung transplantations. J Heart Lung Transplant 2000;19:473-9.

4. Colobran R, Casamitjana N, Roman A, et al. Copy number variation in the CCL4L gene is associated with susceptibility to acute rejection in lung transplantation. Genes Immun 2009;10:254-9.

5. Girnita DM, Webber SA, Zeevi A. Clinical impact of cytokine and growth factor genetic polymorphisms in thoracic organ transplantation. Clin Lab Med 2008;28:423-40, vi.

6. Penninga L, Moller CH, Penninga EI, et al. Antibody induction therapy for lung transplant recipients. Cochrane

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Database Syst Rev 2013:CD008927.

7. Hachem RR, Yusen RD, Chakinala MM, et al. A randomized controlled trial of tacrolimus versus cyclosporine after lung transplantation. J Heart Lung Transplant 2007;26:1012-8.

8. Penninga L, Penninga EI, Moller CH, et al. Tacrolimus versus cyclosporin as primary immunosuppression for lung transplant recipients. Cochrane Database Syst Rev 2013:CD008817.

9. Lafferty KJ, Bootes A, Dart G, et al. Effect of organ culture on the survival of thyroid allografts in mice. Transplantation 1976;22:138-49.

10. Ingulli E. Mechanism of cellular rejection in transplantation. Pediatr Nephrol 2010;25:61-74.

11. De Vito Dabbs A, Hoffman LA, Iacono AT, et al. Are symptom reports useful for differentiating between acute rejection and pulmonary infection after lung transplantation? Heart Lung 2004;33:372-80.

12. Trull A, Steel L, Cornelissen J, et al. Association between blood eosinophil counts and acute cardiac and pulmonary allograft rejection. J Heart Lung Transplant 1998;17:517-24.

13. Speck NE, Schuurmans MM, Murer C, et al. Diagnostic value of plasma and bronchoalveolar lavage samples in acute lung allograft rejection: differential cytology. Respir Res 2016;17:74.

14. Bjortuft O, Johansen B, Boe J, et al. Daily home spirometry facilitates early detection of rejection in single lung transplant recipients with emphysema. Eur Respir J 1993;6:705-8.

15. Otulana BA, Higenbottam T, Ferrari L, et al. The use of home spirometry in detecting acute lung rejection and infection following heart-lung transplantation. Chest 1990;97:353-7.

16. Becker FS, Martinez FJ, Brunsting LA, et al. Limitations of spirometry in detecting rejection after single-lung transplantation. Am J Respir Crit Care Med 1994;150:159-66.

17. Van Muylem A, Melot C, Antoine M, et al. Role of pulmonary function in the detection of allograft dysfunction after heart-lung transplantation. Thorax 1997;52:643-7.

18. Martinu T, Chen DF, Palmer SM. Acute rejection and humoral sensitization in lung transplant recipients. Proc Am Thorac Soc 2009;6:54-65.

19. Millet B, Higenbottam TW, Flower CD, et al. The radiographic appearances of infection and acute rejection of the lung after heart-lung transplantation. Am Rev Respir Dis 1989;140:62-7.

20. Gotway MB, Dawn SK, Sellami D, et al. Acute rejection following lung transplantation: limitations in accuracy of thin-section CT for diagnosis. Radiology 2001;221:207-12.

21. Park CH, Paik HC, Haam SJ, et al. HRCT Features of Acute Rejection in Patients With Bilateral Lung Transplantation: The Usefulness of Lesion Distribution. Transplant Proc 2014;46:1511-6.

22. Hasegawa T, Iacono AT, Yousem SA. The anatomic distribution of acute cellular rejection in the allograft lung. Ann Thorac Surg 2000;69:1529-31.

23. Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant 2007;26:1229-42.

24. Chan CC, Abi-Saleh WJ, Arroliga AC, et al. Diagnostic yield and therapeutic impact of flexible bronchoscopy in lung transplant recipients. J Heart Lung Transplant 1996;15:196-205.

25. McWilliams TJ, Williams TJ, Whitford HM, et al. Surveillance bronchoscopy in lung transplant recipients: risk versus benefit. J Heart Lung Transplant 2008;27:1203-9.

26. Roden AC, Aisner DL, Allen TC, et al. Diagnosis of Acute Cellular Rejection and Antibody-Mediated Rejection on Lung Transplant Biopsies: A Perspective From Members of the Pulmonary Pathology Society. Arch Pathol Lab Med 2017;141:437-44.

27. Yousem SA, Martin T, Paradis IL, et al. Can immunohistological analysis of transbronchial biopsy specimens predict responder status in early acute rejection of lung allografts? Hum Pathol 1994;25:525-9.

28. Chakinala MM, Ritter J, Gage BF, et al. Reliability for grading acute rejection and airway inflammation after lung transplantation. J Heart Lung Transplant 2005;24:652-7.

29. Colombat M, Groussard O, Lautrette A, et al. Analysis of the different histologic lesions observed in transbronchial biopsy for the diagnosis of acute rejection. Clinicopathologic correlations during the first 6 months after lung transplantation. Hum Pathol 2005;36:387-94.

30. Trulock EP, Ettinger NA, Brunt EM, et al. The role of transbronchial lung biopsy in the treatment of lung transplant recipients. An analysis of 200 consecutive procedures. Chest 1992;102:1049-54.

31. Arcasoy SM, Berry G, Marboe CC, et al. Pathologic interpretation of transbronchial biopsy for acute rejection of lung allograft is highly variable. Am J Transplant 2011;11:320-8.

32. Poletti V, Casoni GL, Gurioli C, et al. Lung cryobiopsies: a paradigm shift in diagnostic bronchoscopy? Respirology 2014;19:645-54.

33. Roden AC, Kern RM, Aubry MC, et al. Transbronchial Cryobiopsies in the Evaluation of Lung Allografts: Do the Benefits Outweigh the Risks? Arch Pathol Lab Med 2016;140:303-11.

34. Glanville AR, Aboyoun CL, Havryk A, et al. Severity of lymphocytic bronchiolitis predicts long-term outcome after lung transplantation. Am J Respir Crit Care Med 2008;177:1033-40.

35. Valentine VG, Gupta MR, Weill D, et al. Single-institution study evaluating the utility of surveillance bronchoscopy after lung transplantation. J Heart Lung Transplant 2009;28:14-20.

36. Ahya VN, Douglas LP, Andreadis C, et al. Association between elevated whole blood Epstein-Barr virus (EBV)-encoded RNA EBV polymerase chain reaction and reduced incidence of acute lung allograft rejection. J Heart Lung Transplant 2007;26:839-44.

37. Silkoff PE, Caramori M, Tremblay L, et al. Exhaled nitric oxide in human lung transplantation. A noninvasive marker of acute rejection. Am J Respir Crit Care Med 1998;157:1822-8.

38. Antus B, Csiszer E, Czebe K, et al. Pulmonary infections increase exhaled nitric oxide in lung transplant recipients: a longitudinal study. Clin Transplant 2005;19:377-82.

39. Deborah HY, Anu K, Sharron C, et al. Non-invasive assessment of exhaled biomarkers in lung transplantation. J Breath Res 2011;5:024001.

40. De Vlaminck I, Martin L, Kertesz M, et al. Noninvasive monitoring of infection and rejection after lung transplantation. Proc Natl Acad Sci U S A 2015;112:13336-41.

41. Shino MY, Weigt SS, Saggar R, et al. Usefulness of immune monitoring in lung transplantation using adenosine triphosphate production in activated lymphocytes. J Heart Lung Transplant 2012;31:996-1002.

42. Greenland JR, Wong CM, Ahuja R, et al. Donor-Reactive Regulatory T Cell Frequency Increases During Acute Cellular Rejection of Lung Allografts. Transplantation 2016;100:2090-8.

43. Duong Van Huyen JP, Tible M, Gay A, et al. MicroRNAs as non-invasive biomarkers of heart transplant rejection. Eur Heart J 2014;35:3194-202.

44. Wilflingseder J, Regele H, Perco P, et al. miRNA profiling discriminates types of rejection and injury in human renal allografts. Transplantation 2013;95:835-41.

45. Gharib SA, Edelman JD, Ge L, et al. Acute Cellular Rejection Elicits Distinct MicroRNA Signatures in Airway Epithelium of Lung Transplant Patients. Transplant Direct 2015;1. pii: e44.

46. Khalifah AP, Hachem RR, Chakinala MM, et al. Minimal acute rejection after lung transplantation: a risk for bronchiolitis obliterans syndrome. Am J Transplant 2005;5:2022-30.

47. Aboyoun CL, Tamm M, Chhajed PN, et al. Diagnostic value of follow-up transbronchial lung biopsy after lung rejection. Am J Respir Crit Care Med 2001;164:460-3.

48. Guilinger RA, Paradis IL, Dauber JH, et al. The importance of bronchoscopy with transbronchial biopsy and bronchoalveolar lavage in the management of lung transplant recipients. Am J Respir Crit Care Med 1995;152:2037-43.

49. Sarahrudi K, Estenne M, Corris P, et al. International experience with conversion from cyclosporine to tacrolimus for acute and chronic lung allograft rejection. J Thorac Cardiovasc Surg 2004;127:1126-32.

50. Vitulo P, Oggionni T, Cascina A, et al. Efficacy of tacrolimus rescue therapy in refractory acute rejection after lung transplantation. J Heart Lung Transplant 2002;21:435-9.

51. Reams BD, Musselwhite LW, Zaas DW, et al. Alemtuzumab in the treatment of refractory acute rejection and bronchiolitis obliterans syndrome after human lung transplantation. Am J Transplant 2007;7:2802-8.

52. Benden C, Speich R, Hofbauer GF, et al. Extracorporeal photopheresis after lung transplantation: a 10-year single-center experience. Transplantation 2008;86:1625-7.

53. Hachem R. Antibody-Mediated Lung Transplant Rejection. Curr Respir Care Rep 2012;1:157-61.

54. Westall GP, Snell GI. Antibody-mediated rejection in lung transplantation: fable, spin, or fact? Transplantation 2014;98:927-30.

55. Glanville AR. Antibody-mediated rejection in lung transplantation: myth or reality? J Heart Lung Transplant 2010;29:395-400.

56. Levine DJ, Glanville AR, Aboyoun C, et al. Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2016;35:397-406.

57. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant 2005;24:1710-20.

58. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting

report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014;14:272-83.

59. Felix NJ, Allen PM. Specificity of T-cell alloreactivity. Nat Rev Immunol 2007;7:942-53.

60. Lefaucheur C, Suberbielle-Boissel C, Hill GS, et al. Clinical Relevance of Preformed HLA Donor-Specific Antibodies in Kidney Transplantation. Am J Transplant 2008;8:324-31.

61. Wallace WD, Weigt SS, Farver CF. Update on pathology of antibody-mediated rejection in the lung allograft. Curr Opin Organ Transplant 2014;19:303-8.

62. Tikkanen JM, Singer LG, Kim SJ, et al. De Novo DQ Donor-Specific Antibodies Are Associated with Chronic Lung Allograft Dysfunction after Lung Transplantation. Am J Respir Crit Care Med 2016;194:596-606.

63. Snyder LD, Wang Z, Chen DF, et al. Implications for Human Leukocyte Antigen Antibodies after Lung Transplantation: A 10 year experience in 441 patients. Chest 2013;144:226-33.

64. Ius F, Sommer W, Tudorache I, et al. Early donor-specific antibodies in lung transplantation: risk factors and impact on survival. J Heart Lung Transplant 2014;33:1255-63.

65. Morrell MR, Pilewski JM, Gries CJ, et al. De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. J Heart Lung Transplant 2014;33:1288-94.

66. Le Pavec J, Suberbielle C, Lamrani L, et al. De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome. J Heart Lung Transplant 2016;35:1067-77.

67. Witt CA, Gaut JP, Yusen RD, et al. Acute antibody-mediated rejection after lung transplantation. J Heart Lung Transplant 2013;32:1034-40.

68. Reinsmoen NL, Mirocha J, Ensor CR, et al. A 3 Center Study Reveals New Insights into the Impact of NonHLA Antibodies On Lung Transplantation Outcome. Transplantation 2017;101:1215-21.

69. Roux A, Bendib Le Lan I, Holifanjaniaina S, et al. Antibody-Mediated Rejection in Lung Transplantation: Clinical Outcomes and Donor-Specific Antibody Characteristics. Am J Transplant 2016;16:1216-28.

70. Kauke T, Kneidinger N, Martin B, et al. Bronchiolitis obliterans syndrome due to donor-specific HLA-antibodies. Tissue Antigens 2015;86:178-85.

71. Safavi S, Robinson DR, Soresi S, et al. De novo donor HLA-specific antibodies predict development of

72. Bosanquet JP, Witt CA, Bemiss BC, et al. The impact of pre-transplant allosensitization on outcomes after lung transplantation. J Heart Lung Transplant 2015;34:1415-22.

bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant 2014;33:1273-81.

73. Girnita AL, McCurry KR, Iacono AT, et al. HLA-specific antibodies are associated with high-grade and persistent-recurrent lung allograft acute rejection. J Heart Lung Transplant 2004;23:1135-41.

74. Girnita AL, Duquesnoy R, Yousem SA, et al. HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. Am J Transplant 2005;5:131-8.

75. Kim M, Townsend KR, Wood IG, et al. Impact of pretransplant anti-HLA antibodies on outcomes in lung transplant candidates. Am J Respir Crit Care Med 2014;189:1234-9.

76. Brugiere O, Suberbielle C, Thabut G, et al. Lung transplantation in patients with pretransplantation donor-specific antibodies detected by Luminex assay. Transplantation 2013;95:761-5.

77. Smith JD, Ibrahim MW, Newell H, et al. Pre-transplant donor HLA-specific antibodies: characteristics causing detrimental effects on survival after lung transplantation. J Heart Lung Transplant 2014;33:1074-82.

78. Smith JD, Banner NR, Hamour IM, et al. De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. Am J Transplant 2011;11:312-9.

79. Lobo LJ, Aris RM, Schmitz J, et al. Donor-specific antibodies are associated with antibody-mediated rejection, acute cellular rejection, bronchiolitis obliterans syndrome, and cystic fibrosis after lung transplantation. J Heart Lung Transplant 2013;32:70-7.

80. Westall GP, Paraskeva MA, Snell GI. Antibody-mediated rejection. Curr Opin Organ Transplant 2015;20:492-7.

81. Thomas KA, Valenzuela NM, Reed EF. The perfect storm: HLA antibodies, complement, FcgammaRs, and endothelium in transplant rejection. Trends Mol Med 2015;21:319-29.

82. Mannam VK, Lewis RE, Cruse JM. The fate of renal allografts hinges on responses of the microvascular endothelium. Exp Mol Pathol 2013;94:398-411.

83. AlMahri A, Holgersson J, Alheim M. Detection of complement-fixing and non-fixing antibodies specific for endothelial precursor cells and lymphocytes using flow cytometry. Tissue Antigens 2012;80:404-15.

84. Hachem RR, Tiriveedhi V, Patterson GA, et al. Antibodies

to K-alpha 1 tubulin and collagen V are associated with chronic rejection after lung transplantation. Am J Transplant 2012;12:2164-71.

85. Tait BD, Susal C, Gebel HM, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. Transplantation 2013;95:19-47.

86. Bharat A, Saini D, Steward N, et al. Antibodies to self-antigens predispose to primary lung allograft dysfunction and chronic rejection. Ann Thorac Surg 2010;90:1094-101.

87. Iwata T, Philipovskiy A, Fisher AJ, et al. Anti-type V collagen humoral immunity in lung transplant primary graft dysfunction. J Immunol 2008;181:5738-47.

88. Burlingham W, Wilkes DS, Sullivan JA. Why is the patient out of breath? Collagen V(alpha1) and K-alpha1-tubulin take center stage in lung transplantation. Am J Transplant 2014;14:2201-3.

89. Tiriveedhi V, Gautam B, Sarma NJ, et al. Pre-transplant antibodies to Kalpha1 tubulin and collagen-V in lung transplantation: clinical correlations. J Heart Lung Transplant 2013;32:807-14.

90. Nickerson PW, Rush DN. Antibodies beyond HLA. Am J Transplant 2013;13:831-2.

91. Bharat A, Mohanakumar T. Immune Responses to Tissue-Restricted Nonmajor Histocompatibility Complex Antigens in Allograft Rejection. J Immunol Res 2017;2017:6312514.

92. Fukami N, Ramachandran S, Saini D, et al. Antibodies to MHC class I induce autoimmunity: role in the pathogenesis of chronic rejection. J Immunol 2009;182:309-18.

93. Adeegbe D, Matsutani T, Yang J, et al. CD4(+) CD25(+) Foxp3(+) T regulatory cells with limited TCR diversity in control of autoimmunity. J Immunol 2010;184:56-66.

94. Shah PD, McDyer JF. Viral infections in lung transplant recipients. Semin Respir Crit Care Med 2010;31:243-54.

95. Uhlin M, Mattsson J, Maeurer M. Update on viral infections in lung transplantation. Curr Opin Pulm Med 2012;18:264-70.

96. Papakonstantinou E, Karakiulakis G, Batzios S, et al. Acute exacerbations of COPD are associated with significant activation of matrix metalloproteinase 9 irrespectively of airway obstruction, emphysema and infection. Respir Res 2015;16:78.

97. Morris GP. Anti-HLA Antibodies in Lung Transplantation. OBM Transplantation 2017;1:002.

98. Berry G, Burke M, Anderson C, et al. The pathology of pulmonary antibody mediated rejection: 2012 update from the Pathology Council of ISHLT. J Heart Lung Transplant 2013;32:14-21.

99. Wallace WD, Li N, Andersen CB, et al. Banff study of pathologic changes in lung allograft biopsy specimens with donor-specific antibodies. J Heart Lung Transplant 2016;35:40-8.

100. Roberts JA, Barrios R, Cagle PT, et al. The presence of anti-HLA donor-specific antibodies in lung allograft recipients does not correlate with C4d immunofluorescence in transbronchial biopsy specimens. Arch Pathol Lab Med 2014;138:1053-8.

101. Roden AC, Maleszewski JJ, Yi ES, et al. Reproducibility of Complement 4d deposition by immunofluorescence and immunohistochemistry in lung allograft biopsies. J Heart Lung Transplant 2014;33:1223-32.

102. Westall GP, Snell GI, McLean C, et al. C3d and C4d deposition early after lung transplantation. J Heart Lung Transplant 2008;27:722-8.

103. Appel JZ 3rd, Hartwig MG, Cantu E 3rd, et al. Role of flow cytometry to define unacceptable HLA antigens in lung transplant recipients with HLA-specific antibodies. Transplantation 2006;81:1049-57.

104. Gupta G, Abu Jawdeh BG, Racusen LC, et al. Late antibody-mediated rejection in renal allografts: outcome after conventional and novel therapies. Transplantation 2014;97:1240-6.

105. Marasco SF, Lukas G, McDonald M, et al. Review of ECMO (extra corporeal membrane oxygenation) support in critically ill adult patients. Heart Lung Circ 2008;17 Suppl 4:S41-7.

106. Pena JR, Fitzpatrick D, Saidman SL. Complement-dependent cytotoxicity crossmatch. Methods Mol Biol 2013;1034:257-83.

107. Pei R, Lee JH, Shih NJ, et al. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. Transplantation 2003;75:43-9.

108. Susal C, Opelz G, Morath C. Role and Value of Luminex((R))-Detected HLA Antibodies before and after Kidney Transplantation. Transfus Med Hemother 2013;40:190-5.

109. Liu C, Wetter L, Pang S, et al. Cutoff values and data handling for solid-phase testing for antibodies to HLA: effects on listing unacceptable antigens for thoracic organ transplantation. Hum Immunol 2012;73:597-604.

110. Tambur AR, Herrera ND, Haarberg KM, et al. Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information. Am J Transplant 2015;15:2421-30.

111. Hachem RR, Reinsmoen NL. What is the definition of a clinically relevant donor HLA-specific antibody (DSA)? Am J Transplant 2015;15:299-300.

112. Palmer SM, Davis RD, Hadjiliadis D, et al. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplantation 2002;74:799-804.

113. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. J Heart Lung Transplant 2010;29:973-80.

114. Kulkarni HS, Bemiss BC, Hachem RR. Antibody-mediated Rejection in Lung Transplantation. Curr Transplant Rep 2015;2:316-23.

115. Piazza A, Poggi E, Ozzella G, et al. Post-transplant development of C1q-positive HLA antibodies and kidney graft survival. Clin Transpl 2013:367-75.

116. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. Hum Immunol 2011;72:849-58.

117. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013;369:1215-26.

118. Chin C, Chen G, Sequeria F, et al. Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. J Heart Lung Transplant 2011;30:158-63.

119. Hirohashi T, Uehara S, Chase CM, et al. Complement independent antibody-mediated endarteritis and transplant arteriopathy in mice. Am J Transplant 2010;10:510-7.

120. Kidney Disease: Improving Global Outcomes Transplant Work G. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009;9 Suppl 3:S1-155.

121. Djamali A, Kaufman DB, Ellis TM, et al. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. Am J Transplant 2014;14:255-71.

122. McManigle W, Pavlisko EN, Martinu T. Acute cellular and antibody-mediated allograft rejection. Semin Respir Crit Care Med 2013;34:320-35.

123. Bansal SB. Rituximab use in late antibody-mediated rejection. Indian J Nephrol 2016;26:315-6.

124. Roberts DM, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. Transplantation 2012;94:775-83.

125. Ejaz NS, Alloway RR, Halleck F, et al. Review of bortezomib treatment of antibody-mediated rejection in renal transplantation. Antioxid Redox Signal 2014;21:2401-18.

126. Walsh RC, Alloway RR, Girnita AL, et al. Proteasome inhibitor-based therapy for antibody-mediated rejection. Kidney Int 2012;81:1067-74.

127. Walsh RC, Brailey P, Girnita A, et al. Early and late acute antibody-mediated rejection differ immunologically and in response to proteasome inhibition. Transplantation 2011;91:1218-26.

128. Yang KS, Jeon H, Park Y, et al. Use of bortezomib as anti-humoral therapy in kidney transplantation. J Korean Med Sci 2014;29:648-51.

129. Flechner SM, Fatica R, Askar M, et al. The role of proteasome inhibition with bortezomib in the treatment of antibody-mediated rejection after kidney-only or kidney-combined organ transplantation. Transplantation 2010;90:1486-92.

130. Moreau P, Pylypenko H, Grosicki S, et al. Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study. Lancet Oncol 2011;12:431-40.

131. Locke JE, Magro CM, Singer AL, et al. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. Am J Transplant 2009;9:231-5.

132. Yeung MY, Gabardi S, Sayegh MH. Use of polyclonal/monoclonal antibody therapies in transplantation. Expert Opin Biol Ther 2017;17:339-52.

Chronic lung allograft dysfunction phenotypes and treatment

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Abstract: Chronic lung allograft dysfunction (CLAD) remains a major hurdle limiting long-term survival post lung transplantation. Given the clinical heterogeneity of CLAD, recently two phenotypes of CLAD have been defined [bronchiolitis obliterans syndrome (BOS) vs. restrictive allograft syndrome (RAS) or restrictive CLAD (rCLAD)]. BOS is characterized by an obstructive pulmonary function, air trapping on CT and obliterative bronchiolitis (OB) on histopathology, while RAS/rCLAD patients show a restrictive pulmonary function, persistent pleuro-parenchymal infiltrates on CT and pleuroparenchymal fibro-elastosis on biopsies. Importantly, the patients with RAS/rCLAD have a severely limited survival post diagnosis of 6–18 months compared to 3–5 years after BOS diagnosis. In this review, we will review historical evidence for this heterogeneity and we will highlight the clinical, radiological, histopathological characteristics of both phenotypes, as well as their risk factors. Treatment of CLAD remains troublesome, nevertheless, we will give an overview of different treatment strategies that have been tried with some success. Adequate phenotyping remains difficult but is clearly needed for both clinical and scientific purposes.

Keywords: Lung transplantation; chronic rejection; bronchiolitis obliterans syndrome (BOS); restrictive allograft syndrome (RAS); chronic lung allograft dysfunction (CLAD)

Introduction

Although survival has improved spectacularly following lung transplantation, it still limbs behind that after other solid organ transplantations (1). Although improvement in surgical techniques and clinical expertise led to an improved in initial survival, long-term overall outcome remains poor. Graft failure and chronic lung allograft dysfunction (CLAD) are the major culprits for this inferior long-term outcome (2). CLAD has been introduced recently as an overarching term encompassing all forms of chronic (>3 weeks) pulmonary function decline. Next to pulmonary function decline with a known cause (either graft-related, i.e., acute rejection, recurrence of native disease, infection, suture problems; or non-graft related, i.e., obesitas, pleural fluid, diaphragm dysfunction), there is also a large proportion of patients in whom no clear cause can be identified for the decline in pulmonary function which is therefore assumed to be due to chronic rejection. Within this review, we will describe historic and current evidence for CLAD classification and its clinical implications (diagnosis, pathology, radiology, risk factors and mechanisms) with a particular focus on treatment.

History

Historically, the term bronchiolitis obliterans syndrome (BOS) has been universally linked with chronic rejection post-transplant. BOS was defined as a persistent, obstructive decrease in forced expiratory volume in 1 second (FEV1) with at least 20% compared to the mean of the two best post-transplant values, in the absence of other

identifiable causes such as acute rejection, infection, suture problems… Further stratification was made according to the relative decrease in FEV_1 and consequently a grading system was introduced being BOS1 (FEV_1 66–80% of best), BOS2 (FEV 50–65% of best) and BOS3 (<50% of best). BOS was thought to be a functional reflection of obliterative bronchiolitis (OB). OB was considered to be the pathological hallmark of chronic rejection, but can also be found in other conditions such as pulmonary graft versus host disease after hematopoietic stem cell transplantation, auto-immune disorders especially rheumatoid arthritis, inhalation of toxins like sulfur mustard or as a post-infectious complication following childhood viral infection (3). OB is a pathological scarring or filling of the airway lumen with collagenous matrix leading to airflow limitation. The clinical definition of BOS based on serial pulmonary function measurements was deemed necessary given the poor sensitivity and specificity to diagnose OB on transbronchial biopsies (4). However, already in the initial pathological descriptions of explant lungs of patients suffering from chronic rejection some discrepancies were observed, such as the occurrence of a restrictive pulmonary function decline and significant pleural thickening (5,6). Nevertheless, in the following decades, the term BOS was universally utilized when referring to chronic rejection. The first elements to break this dogma came in 2003 when Gerhardt *et al.* found a proportion of patients with established BOS, who improved their pulmonary function upon azithromycin treatment (7). In some patients, this FEV_1 improvement was so pronounced that criteria for BOS were no longer fulfilled. This was confirmed by several other groups (8,9) and led to the first proposition of phenotypes of chronic rejection, leading to a novel phenotype called neutrophilic reversible allograft dysfunction or azithromycin responsive allograft dysfunction (10), which is nowadays considered as a reversible cause of CLAD and therefore is no longer thought to be a manifestation of chronic rejection (11). It was only in 2010 that a restrictive pulmonary function defect came apparent when Woodrow and colleagues defined a group of patients with so called 'restrictive BOS' (12). However, this description had no clinical implications (i.e., no survival difference) and therefore it was only in 2011 when Sato *et al.* identified a restrictive allograft syndrome (RAS) in patients with a decline in total lung capacity and infaust prognosis that general interest was aroused for what was thought to be a novel manifestation of chronic rejection (13). Typically, these patients presented with a

restrictive pulmonary function, persistent CT infiltrates and most interestingly inferior survival compared to the obstructive (BOS) patients. Since literature on known causes of CLAD is rare, we will emphasize and contrast BOS to RAS and compare clinical characteristics, with special emphasis on treatment.

BOS

Diagnosis, radiology and pathology

BOS remains the most common phenotype of chronic rejection (65–75%). Typical characteristics include an obstructive pulmonary function defect and air trapping/mosaic attenuation on expiratory CT. Median survival after diagnosis is between 3–5 years. However, even within BOS there is significant heterogeneity: patients with an early (<2 years post-transplant) or a high grade onset (FEV_1 decline >35%) have inferior survival compared to patients with late and low grade onset (14). Analysis of explant specimens at redo transplantation has revealed OB in all BOS lungs (15), and the lesions seem to be segmental with 40–60% of the small airways appearing obstructed as of generation 6 on (16), which may explain the obstructive pulmonary function. OB is thought to be the end-result of persistent damage to the bronchial epithelium leading to an excessive inflammatory response, leading to local (myo-) fibroblast recruitment, fibrosis and ultimately complete obliteration of the airway lumen by fibrotic matrix.

Risk factors and mechanisms of BOS

Many risk factors for BOS have been identified such as acute rejection [specifically acute rejections associated with pulmonary function decline (17)], lymphocytic bronchiolitis, infection and colonization with micro-organisms (i.e., *Pseudomonas aeruginosa* and *Aspergillus fumigatus*), donor and recipient genetics, primary graft dysfunction, particulate matter and presence of HLA antibodies, or antibodies to self-antigens (18). Especially regarding the latter, progress has been made the last years. De novo development of donor specific antibodies occurs frequently (35–60%) and is independently associated with CLAD (19,20). Similarly, antibodies to self-antigens (like K-α1 tubulin and collagen V) have been demonstrated to increase the risk for subsequent BOS development (21).

Since the mechanisms of BOS remain mostly elusive, novel evidence is accumulating with the use of the mice

orthotopic lung transplant model. Depending on the type of mismatch, immunosuppression and the duration of follow-up, lesions compatible with OB can be found in transplanted mice lung. Given the advantage of genetics knockouts in mice and possibility of invasive sampling, this model is an excellent set-up to study underlying mechanisms. For example, it was shown that progressive loss of self-tolerance through epitope spreading promotes airway fibrosis (22). In another experiment, it has been shown that the murine lung allograft fibrosis originates mostly from the donor (23). However, these results cannot be directly extrapolated from mice to men as in humans, 32% of OB lesions are occupied by recipient and not donor fibroblasts (24). Moreover, human OB mostly develops in small airways, yet mice lack small airways, which is an additional problem to overcome. Therefore, experimental research has also focused on *in vitro* culture of bronchial epithelial cells, which showed that transition of epithelial cells to a mesenchymal phenotype can contribute to the fibroblast accumulation in OB lesions (25). Interestingly, Pseudomonas can significantly aggravate this so called epithelial-mesenchymal transition (EMT), which is important given that colonization with pseudomonas occurs frequently post-transplant and is independently associated with a higher prevalence of BOS (26).

For decades, the search has been ongoing to identify an appropriate marker for BOS. Given the heterogeneous nature of CLAD, it comes to no surprise that at this moment, there are no universally applied biomarkers for BOS diagnosis. Broncho-alveolar lavage (BAL) analysis may provide insights in the lung micro-environment (27). Using BAL, the first important markers for BOS came to light, which included neutrophils and markers of neutrophil activation (CXL-8, MMP-9) (28). Later, however, it became clear that patients with elevated BAL neutrophilia and IL-8 are those who display the best response to treatment with neomacrolides (most commonly azithromycin which was denominated azithromycin responsive allograft dysfunction or neutrophilic responsive allograft syndrome, see above). The same was later seen with BAL IL-17, which has been implicated in BOS. IL-17 is a major pro-inflammatory molecule inducing the release of IL-8, but is also implicated in the response to self-antigens. However, IL-17 staining in the lamina propria later revealed no difference in BOS compared to stable patients (29), while orthotopic lung transplantation in major mismatch mouse strains did not reveal a difference between wildtype and IL-17 knock-out mice (30). In patients with lymphocytic bronchiolitis, who were treated with azithromycin, IL-17 positive cells

disappear from the lamina propria and FEV_1 increases (31). Nevertheless, these patients may later still develop BOS, without IL-17 involvement. Evidence from other groups nevertheless suggest an important role for IL-17 in CLAD as treatment with an anti-IL-17 antibody or with halofuginone (which reduces IL-17), may attenuate features of chronic rejection in a murine transplant model (32,33).

Overall, none of the historically identified proteins seem to be a good biomarker for BOS development. In fact, a recent BAL cytokine and chemokine analysis, could not detect any molecule that was differentially regulated between stable (non-rejecting) patients and patients with BOS (34). Consequently, some groups have tried to identify blood markers blood for BOS development, but so far none have proven to be very sensitive and specific.

Treatment

The widespread use of the neomacrolides has significantly impacted CLAD incidence and long-term survival. In fact, a randomized placebo controlled prevention trial with azithromycin initiated at hospital discharge following transplantation has shown that patients taking azithromycin demonstrate better pulmonary function, as well as decreased BAL neutrophilia and lower CLAD prevalence (35). A recent post-hoc analysis of this trial revealed that these long-term beneficial effects persisted and that azithromycin was able to significantly postpone the development of CLAD (36). Treatment with macrolides in established CLAD also seems to be an adequate treatment option (37) but given the rarity of randomized controlled trials in this field, we do not know if either prophylactic or targeted treatment is superior. An expert task force concluded that currently available therapies have not shown a significant benefit in preventing or treating BOS, although investigation of possible underlying gastro-oesophageal reflux and a trial with macrolides in BOS is recommended (18). Some other therapies have shown promise in smaller, mostly single-center studies which are briefly discussed below.

Despite attenuation of neutrophils by azithromycin, in a subset of patients elevated airway neutrophilia later can redevelop. These patients usually present with a colonized graft (mostly pseudomonas) and demonstrate inferior survival compared to patients without neutrophilia (38). Interestingly, IL-1α is increased in BAL of those patients indicating that these alarmins might play an important role in the pathophysiology of BOS (39). Macrolide treatment does not seem to affect these patients (40). However

extracorporeal photopheresis (ECP), a leukapheresis-based procedure, was beneficial (41), and seems to be mainly an adequate treatment for patient with macrolide resistant airway neutrophilia (42). Part of this beneficial effect can be explained by effects of ECP on reducing inflammatory cytokines, chemokines and donor specific antibodies (43).

Montelukast, a cysteinyl leukotriene inhibitor is another possible treatment for BOS. A case series demonstrated a less pronounced decrease in pulmonary function in patients treated with montelukast compared to never treated patients (44). In a randomized placebo-controlled trial, montelukast was shown to be beneficial especially BOS stage 1 compared to placebo, but in later BOS stages no beneficial effects were seen (Ruttens *et al.* submitted).

As a last option for BOS, redo transplantation can be considered, amounting to about 5% of the total number of transplantations being performed annually. Although survival is not as good compared to a primary transplantation, for a well-selected group of patients redo transplantation may be the only option to improve outcome and quality of life (45). Given the scarcity of donor organs, this is not an option offered at every transplant center.

Restrictive CLAD (rCLAD)

Diagnosis, radiology and pathology

Besides the most commonly known BOS phenotype, the rCLAD seems to be gaining a lot of interest lately. Diagnostics remains troublesome at the moment. The initial report by Sato *et al.* used a decline in TLC of at least 10% to diagnose patients suffering from a restrictive pulmonary function defect (13), while Todd *et al.* used a FVC decrease >20% (46) and Verleden *et al.* used a combination of TLC and FEV_1/FVC (47). The common denominator in all these patients is the presence of persistent pleuroparenchymal infiltrates on CT imaging. Therefore Suhling *et al.* proposed to use a combination of pulmonary infiltrates on CT and pulmonary function measurements, specifically a TLC decrease >20% (48). In single-lung transplanted recipients, accurate rCLAD diagnosis is more complicated, given the confounding effect of the native lung, but a FVC decrease >20% was also associated with a poor outcome in a multi-center cohort study (49). This poor outcome is also a common denominator in all aforementioned studies: independent of the criteria used to diagnose restriction, outcome was worse in patients with a restrictive (rCLAD) *vs.* an obstructive (BOS) pulmonary function defect, with a

median post-diagnosis survival of 6–18 months in rCLAD compared to 3–5 years in BOS (50). Prevalence of rCLAD is quite similar across different centers with 25–35% of CLAD patients affected (50). It is important to note that this classification is not absolute and that patients can evolve at any time during their post-transplant course from BOS to RAS or vice versa. Most often, patients evolve from an obstructive to a restrictive form of CLAD, however the opposite has also been described (13). Evolution from BOS to rCLAD is very difficult to diagnose, given the underlying severe obstruction, but it does not seem to imply a worse prognosis (51). A representative case with an initial BOS diagnosis is shown in *Figure 1*, as well as his evolution towards later rCLAD.

Nowadays diagnostic guidelines for rCLAD are lacking, CT is not implemented as a diagnostic criterium for rCLAD. However, rCLAD typically shows significantly different radiology compared to BOS, as there are signs of (sub)pleural thickening and pleuroparenchymal infiltrates. The land-mark study of Sato *et al.* showed an apical predominance in a significant subset of patients (13), which was in line with the earlier observation of upper-lobe dominant fibrosis post-lung transplantation (52). However, we recently demonstrated that there are also patients with diffuse or basal-dominated infiltrates on CT and interestingly, these patients had a worse outcome compared to patients with apical dominated fibrosis (51), while the degree of consolidation, ground glass or reticulation did not correlate with survival post diagnosis (53). CT could also be used as alternative tool to diagnose rCLAD, as lungs have significantly lower lung volume compared to baseline, while the volume of lungs in BOS remains stable or even increases (54). This could provide an easy to interpret, add-on tool to diagnose rCLAD when pulmonary function tests are inconclusive. Thorough investigation of rCLAD explant lungs using CT and microCT demonstrated disappearing airways on CT, with OB in 30–40% of the remaining airways. Further, microCT showed a decrease in the number of terminal bronchioles (the last conducting airway before the alveoli). Therefore, this indicates that the airways are also involved in rCLAD, although the proportion of OB lesions was not that high as in pure BOS (55). Next to this airway involvement, the alveoli looked completely different reflecting interstitial and/or alveolar fibrosis.

On pathological examination pleuroparenchymal fibro-elastosis is the most common histological pattern of rCLAD (56). Molecular analysis of this alveolar fibro-elastosis pattern revealed that the initial changes are a non-
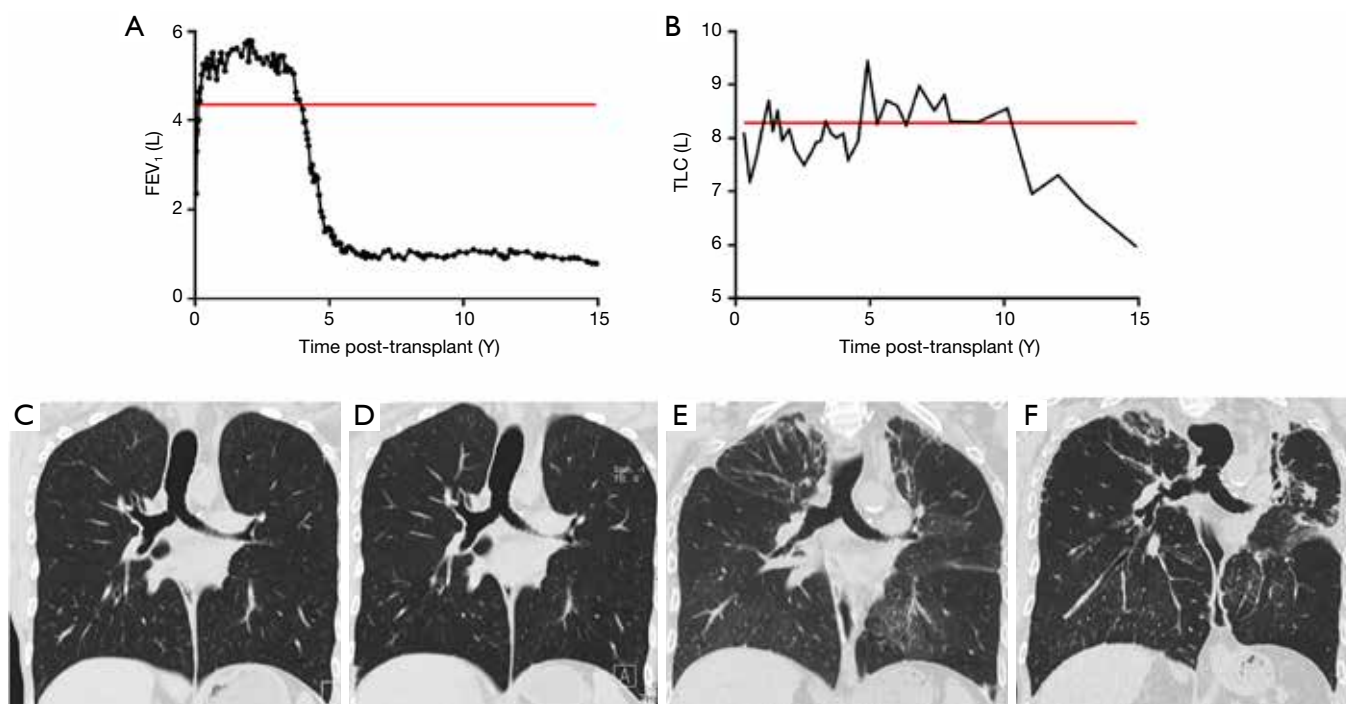
Figure 1 A patient underwent heart lung transplantation for Eisenmenger's syndrome with an initial uneventful follow-up post discharge who developed BOS. 5 years post-transplantation (pulmonary function evolution in A), but without decrease in TLC (B). However 10 years post-transplantation there was a sudden TLC drop (red line indicates 10% decrease) and therefore diagnosis was changed to rCLAD/RAS. CT evolution is shown in panel 1C-D-E-F. Initially, the patient had a normal CT (C), which remained unchanged after BOS diagnosis (D). However, when the decrease in TLC was found, persistent apical infiltrates were seen on CT (E), which deteriorated at the last CT before successful redo transplantation (F). The histological analysis of this explant lung confirmed rCLAD diagnosis as a pattern of pleuroparenchymal fibro-elastosis and OB was observed.

specific fibrin reaction to a yet unknown injury, which progresses to a failed attempt to resolve this, resulting in manifest fibro-elastosis (57). The Melbourne group first described such a pattern consistent with acute fibrinous and organising pneumonia (AFOP) on transbronchial biopsy, which is also associated with a non-obstructive pulmonary function decline, persistent infiltrates and poor outcome in surviving patients (58). Thus, AFOP and rCLAD are likely to represent two entities (acute-chronic) of the same fibrotic spectrum.

Risk factors and mechanisms

The body of evidence for risk factors specific for rCLAD is not that robust as for BOS, although it seems that many risk factors are similar between both phenotypes. Indeed, acute cellular rejection, lymphocytic bronchiolitis, colonization with Pseudomonas, infection, and BAL neutrophilia were equally important for later BOS and rCLAD (59). Of interest BAL and blood eosinophilia, a cell that is mostly discarded in lung transplantation because of its low relative abundance, shows a strong association with subsequent development of rCLAD (60). Moreover, in patients diagnosed with rCLAD, BAL and blood eosinophilia are also able to dissect those rCLAD patients with the worst prognosis, indicating that eosinophilia could serve as an easy marker for rCLAD development and prognosis following diagnosis (51). Other studies focused on particular (inflammatory) cytokines and chemokines. For example, specific increase in pro-inflammatory alveolar alarmins (61), IL-6 and IP-10 (34) could be important in the pathophysiology of rCLAD. An immunohistochemistry study of rCLAD explant lungs revealed pronounced inflammation, with a significant increase in macrophages, neutrophils, mast-cells, eosinophils, CD8 T-cells and interestingly B-cells. These B-cells were organized in

lymphoid follicles, which is a common finding in other chronic respiratory diseases (62). Given this presence of lymphoid follicles, it comes as no surprise that immunoglobulin levels were also increased in rCLAD (63). Therefore, this raises the question to which extent rCLAD overlaps with chronic antibody-mediated rejection (AMR). AMR is an acute or subacute form of graft injury wherein antibodies against donor human leukocyte antigens cause characteristic lung histology (for instance neutrophilic capillaritis) with or without evidence of endothelial C4d staining (64). The presence of HLA antibodies seems to be more associated with rCLAD compared to BOS which is in line with the hypothesis of (at least part) overlap (65).

Of interest is also that in BAL, VEGF levels are decreased in rCLAD patients (34), which is in line with the hypothesis that the capillary network is of importance, which was also demonstrated in a descriptive pathological study (66). In contrast, the lymphatics do not seem to be altered in rCLAD, which is surprising given the predominant distribution (pleural and septal) of fibrosis in rCLAD (67). Despite these interesting observations, more research is needed to elucidate the pathophysiological mechanisms in rCLAD.

Treatment

Similar as in BOS, treatment of rCLAD remains troublesome. The disease course is very unpredictable, given that the disease evolution follows a stepwise pattern of decline: an acute phase characterised by acute lung injury (diffuse alveolar damage, DAD), followed by a resolution stage, during which fibrosis further develops (68). Therefore, patients who at first seem stable can evolve rapidly to a more severe (sometimes even life-threatening) disease stage requiring urgent redo transplantation or death. In that respect, it is important to realize that survival after redo transplantation for rCLAD is inferior compared to BOS, which by itself is already worse compared to survival after primary transplant [3-year survival of 67% in BOS and 33% in rCLAD (69)]. Also, CLAD more frequently redevelops following redo transplantation for rCLAD, again limiting long-term survival. Given these disappointing results, anti-fibrotic treatment may be a good option, based on the positive experience in IPF patients, where it has been shown to slow down the FVC decline (70). Although the experience in treating rCLAD patients is limited at this moment, case reports of successful treatment with pirfenidone (71) and nintedanib (72) described stabilization of the disease, which may be considered a success given

the bad prognosis after diagnosis. Nevertheless, no large cohorts have been described so far and therefore more evidence is needed before antifibrotics can be introduced in general clinical practice. ECP therapy does not seem to be able to slow down rCLAD progression and therefore does not seem a viable option (42). Another drug with potential to slow down disease progression is alemtuzumab (Campath-1H), an antagonist of CD52 which is expressed on B-cells, lymphocytes, dendritic cells and monocytes. This drug was found to improve interstitial changes and lung function in four patients who likely had rCLAD (73), while it was also described in successful treatment of persistent acute rejection (74). Another approach of treating rCLAD might be trying to decrease or erase HLA antibodies by using plasmapheresis, intravenous immunoglobulins and rituximab, which has shown to be partly successful in at least reducing the antibody titre (75). However, true efficacy in treating or stabilizing rCLAD remains unknown. Therefore, at present, there are little effective therapeutic options for rCLAD. Hopefully, a better understanding of the pathophysiological mechanisms will lead to a rapid and efficient therapeutic strategy which is desperately needed given the poor outcome of these patients.

Conclusions

BOS and rCLAD are separate entities within CLAD, with their own clinical, radiological and pathological characteristics (see *Figure 2* for illustration). To what degree these syndromes differ is at this moment unknown. Given the overlap in risk factors and the fact that OB lesions are detected in both syndromes, and the possible evolution of one syndrome to another, there is likely at least some degree of overlap between BOS and rCLAD. More importantly, rigorous identification of the different phenotypes is clearly needed for both clinical and scientific purposes. Further advance in this field is limited by the absence of uniform diagnostic criteria for rCLAD, which makes the design of multicentre studies nearly impossible. Yet, given the rather low incidence of rCLAD in individual centers, monocentric studies are currently hampered by the number of patients that can be included. Only by doing so, we can adequately power and design clinical trials which are desperately needed given the disappointing outcome after lung transplantation compared to other solid organ transplantations. These different phenotypes are nonetheless an indication that the future will probably lie in individualized therapy, needed to further improve survival.
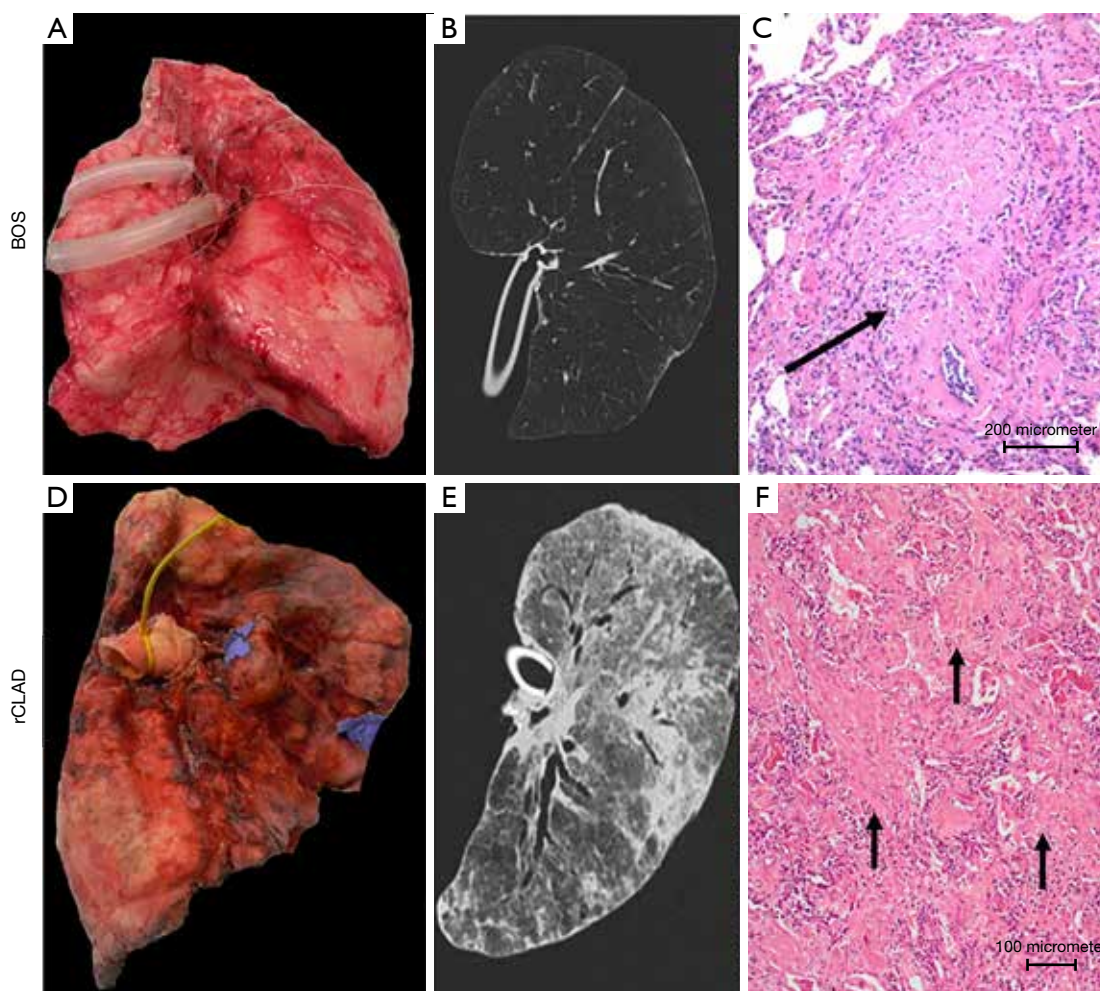
Figure 2 Comparison between BOS and rCLAD. Gross image of a BOS lung (A), with the CT showing typical hyperinflation (B), while the explant lung specimen shows obliterative bronchiolitis (arrow) (C). Gross lung image of RAS/rCLAD lung (D), with the CT showing ground glass and reticulation (E) and the pathology showing newly formed fibrosis (arrows). H&E stainings are used in C and F.

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Footnote

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References

1. Opelz G, Döhler B, Ruhenstroth A, et al. The collaborative transplant study registry. Transplant Rev (Orlando) 2013;27:43-5.
2. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.
3. Barker AF, Bergeron A, Rom WN, et al. Obliterative bronchiolitis. N Engl J Med 2014;370:1820-8.
4. Chamberlain D, Maurer J, Chaparro C, et al. Evaluation of transbronchial lung biopsy specimens in the diagnosis of bronchiolitis obliterans after lung transplantation. J Heart Lung Transplant 1994;13:963-71.

5. Yousem SA, Burke CM, Billingham ME. Pathologic pulmonary alterations in long-term human heart-lung transplantation. Hum Pathol 1985;16:911-23.

6. Burke CM, Theodore J, Dawkins KD, et al. Post-transplant obliterative bronchiolitis and other late lung sequelae in human heart-lung transplantation. Chest 1984;86:824-9.

7. Gerhardt SG, McDyer JF, Girgis RE, et al. Maintenance azithromycin therapy for bronchiolitis obliterans syndrome: results of a pilot study. Am J Respir Crit Care Med 2003;168:121-5.

8. Verleden GM, Vanaudenaerde BM, Dupont LJ, et al. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2006;174:566-70.

9. Gottlieb J, Szangolies J, Koehnlein T, et al. Long-term azithromycin for bronchiolitis obliterans syndrome after lung transplantation. Transplantation 2008;85:36-41.

10. Vanaudenaerde BM, Meyts I, Vos R, et al. A dichotomy in bronchiolitis obliterans syndrome after lung transplantation revealed by azithromycin therapy. Eur Respir J 2008;32:832-43.

11. Verleden GM, Raghu G, Meyer KC, et al. A new classification system for chronic lung allograft dysfunction. J Heart Lung Transplant 2014;33:127-33.

12. Woodrow JP, Shlobin OA, Barnett SD, et al. Comparison of bronchiolitis obliterans syndrome to other forms of chronic lung allograft dysfunction after lung transplantation. J Heart Lung Transplant 2010;29:1159-64.

13. Sato M, Waddell TK, Wagnetz U, et al. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. J Heart Lung Transplant 2011;30:735-42.

14. Finlen Copeland CA, Snyder LD, Zaas DW, et al. Survival after bronchiolitis obliterans syndrome among bilateral lung transplant recipients. Am J Respir Crit Care Med 2010;182:784-9.

15. Martinu T, Howell DN, Davis RD, et al. Pathologic correlates of bronchiolitis obliterans syndrome in pulmonary retransplant recipients. Chest 2006;129:1016-23.

16. Verleden SE, Vasilescu DM, Willems S, et al. The site and nature of airway obstruction after lung transplantation. Am J Respir Crit Care Med 2014;189:292-300.

17. Davis WA, Finlen Copeland CA, Todd JL, et al. Spirometrically significant acute rejection increases the risk for BOS and death after lung transplantation. Am J Transplant 2012;12:745-52.

18. Meyer KC, Raghu G, Verleden GM, et al. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J 2014;44:1479-503.

19. Le Pavec J, Suberbielle C, Lamrani L, et al. De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome. J Heart Lung Transplant 2016;35:1067-77.

20. Tikkanen JM, Singer LG, Kim SJ, et al. De Novo DQ Donor-Specific Antibodies Are Associated with Chronic Lung Allograft Dysfunction after Lung Transplantation. Am J Respir Crit Care Med 2016;194:596-606.

21. Angaswamy N, Tiriveedhi V, Sarma NJ, et al. Interplay between immune responses to HLA and non-HLA self-antigens in allograft rejection. Hum Immunol 2013;74:1478-85.

22. Subramanian V, Ramachandran S, Banan B, et al. Immune response to tissue-restricted self-antigens induces airway inflammation and fibrosis following murine lung transplantation. Am J Transplant 2014;14:2359-66.

23. Mimura T, Walker N, Aoki Y, et al. Local origin of mesenchymal cells in a murine orthotopic lung transplantation model of bronchiolitis obliterans. Am J Pathol 2015;185:1564-74.

24. Bröcker V, Länger F, Fellous TG, et al. Fibroblasts of recipient origin contribute to bronchiolitis obliterans in human lung transplants. Am J Respir Crit Care Med 2006;173:1276-82.

25. Borthwick LA, Suwara MI, Carnell SC, et al. Pseudomonas aeruginosa induced airway epithelial injury drives fibroblast activation: a mechanism in chronic lung allograft dysfunction. Am J Transplant 2016;16:1751-65.

26. Botha P, Archer L, Anderson RL, et al. Pseudomonas aeruginosa colonization of the allograft after lung transplantation and the risk of bronchiolitis obliterans syndrome. Transplantation 2008;85:771-4.

27. Kennedy VE, Todd JL, Palmer SM. Bronchoalveolar lavage as a tool to predict, diagnose and understand bronchiolitis obliterans syndrome. Am J Transplant 2013;13:552-61.

28. Riise GC, Williams A, Kjellström C, et al. Bronchiolitis obliterans syndrome in lung transplant recipients is associated with increased neutrophil activity and decreased antioxidant status in the lung. Eur Respir J 1998;12:82-8.

29. Verleden SE, Vos R, Vandermeulen E, et al. Involvement of interleukin-17 during lymphocytic bronchiolitis in lung transplant patients. J Heart Lung Transplant 2013;32:447-53.

30. Yamada Y, Vandermeulen E, Heigl T, et al. The role of

recipient derived interleukin-17A in a murine orthotopic lung transplant model of restrictive chronic lung allograft dysfunction. Transpl Immunol 2016;39:10-7.

31. Vos R, Verleden SE, Ruttens D, et al. Azithromycin and the treatment of lymphocytic airway inflammation after lung transplantation. Am J Transplant 2014;14:2736-48.

32. Oishi H, Martinu T, Sato M, et al. Halofuginone Treatment reduces interleukin-17A and ameliorates features of chronic lung allograft dysfunction in a mouse orthotopic lung transplant model. J Heart Lung Transplant 2016;35:518-27.

33. Fan L, Benson HL, Vittal R, et al. Neutralizing IL-17 prevents obliterative bronchiolitis in murine orthotopic lung transplantation. Am J Transplant 2011;11:911-22.

34. Verleden SE, Ruttens D, Vos R, et al. Differential cytokine, chemokine and growth factor expression in phenotypes of chronic lung allograft dysfunction. Transplantation 2015;99:86-93.

35. Vos R, Vanaudenaerde BM, Verleden SE, et al. A randomised controlled trial of azithromycin to prevent chronic rejection after lung transplantation. Eur Respir J 2011;37:164-72.

36. Ruttens D, Verleden SE, Vandermeulen E, et al. Prophylactic Azithromycin Therapy After Lung Transplantation: Post hoc Analysis of a Randomized Controlled Trial. Am J Transplant 2016;16:254-61.

37. Corris PA, Ryan VA, Small T, et al. A randomised controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome (BOS) post lung transplantation. Thorax 2015;70:442-50.

38. Vandermeulen E, Verleden SE, Ruttens D, et al. BAL neutrophilia in azithromycin-treated lung transplant recipients: Clinical significance. Transpl Immunol 2015;33:37-44.

39. Suwara MI, Vanaudenaerde BM, Verleden SE, et al. Mechanistic differences between phenotypes of chronic lung allograft dysfunction after lung transplantation. Transpl Int 2014;27:857-67.

40. Bellon H, Vandermeulen E, Mathyssen C, et al. Interleukin-1α induced release of interleukin-8 by human bronchial epithelial cells in vitro: assessing mechanisms and possible treatment options. Transpl Int 2017;30:388-97.

41. Benden C, Speich R, Hofbauer GF, et al. Extracorporeal photopheresis after lung transplantation: a 10-year single-center experience. Transplantation 2008;86:1625-7.

42. Greer M, Dierich M, De Wall C, et al. Phenotyping established chronic lung allograft dysfunction predicts extracorporeal photopheresis response in lung transplant

patients. Am J Transplant 2013;13:911-8.

43. Baskaran G, Tiriveedhi V, Ramachandran S, et al. Efficacy of extracorporeal photopheresis in clearance of antibodies to donor-specific and lung-specific antigens in lung transplant recipients. J Heart Lung Transplant 2014;33:950-6.

44. Verleden GM, Verleden SE, Vos R, et al. Montelukast for bronchiolitis obliterans syndrome after lung transplantation: a pilot study. Transpl Int 2011;24:651-6.

45. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-first adult lung and heart-lung transplant report--2014; focus theme: retransplantation. J Heart Lung Transplant 2014;33:1009-24.

46. Todd JL, Jain R, Pavlisko EN, et al. Impact of forced vital capacity loss on survival after the onset of chronic lung allograft dysfunction. Am J Respir Crit Care Med 2014;189:159-66.

47. Verleden GM, Vos R, Verleden SE, et al. Survival determinants in lung transplant patients with chronic allograft dysfunction. Transplantation 2011;92:703-8.

48. Suhling H, Dettmer S, Greer M, et al. Phenotyping Chronic Lung Allograft Dysfunction Using Body Plethysmography and Computed Tomography. Am J Transplant 2016;16:3163-70.

49. DerHovanessian A, Todd JL, Zhang A, et al. Validation and Refinement of Chronic Lung Allograft Dysfunction Phenotypes in Bilateral and Single Lung Recipients. Ann Am Thorac Soc 2016;13:627-35.

50. Verleden SE, Ruttens D, Vandermeulen E, et al. Restrictive chronic lung allograft dysfunction: Where are we now? J Heart Lung Transplant 2015;34:625-30.

51. Verleden SE, Ruttens D, Vandermeulen E, et al. Predictors of survival in restrictive chronic lung allograft dysfunction after lung transplantation. J Heart Lung Transplant 2016;35:1078-84.

52. Pakhale SS, Hadjiliadis D, Howell DN, et al. Upper lobe fibrosis: a novel manifestation of chronic allograft dysfunction in lung transplantation. J Heart Lung Transplant 2005;24:1260-8.

53. Verleden SE, de Jong PA, Ruttens D, et al. Functional and computed tomographic evolution and survival of restrictive allograft syndrome after lung transplantation. J Heart Lung Transplant 2014;33:270-7.

54. Saito T, Horie M, Sato M, et al. Low-dose computed tomography volumetry for subtyping chronic lung allograft dysfunction. J Heart Lung Transplant 2016;35:59-66.

55. Verleden SE, Vasilescu DM, McDonough JE, et al.

Linking clinical phenotypes of chronic lung allograft dysfunction to changes in lung structure. Eur Respir J 2015;46:1430-9.

56. Ofek E, Sato M, Saito T, et al. Restrictive allograft syndrome post lung transplantation is characterized by pleuroparenchymal fibroelastosis. Mod Pathol 2013;26:350-6.

57. Jonigk D, Izykowski N, Rische J, et al. Molecular Profiling in Lung Biopsies of Human Pulmonary Allografts to Predict Chronic Lung Allograft Dysfunction. Am J Pathol 2015;185:3178-88.

58. Paraskeva M, McLean C, Ellis S, et al. Acute fibrinoid organizing pneumonia after lung transplantation. Am J Respir Crit Care Med 2013;187:1360-8.

59. Verleden SE, Ruttens D, Vandermeulen E, et al. Bronchiolitis obliterans syndrome and restrictive allograft syndrome: do risk factors differ? Transplantation 2013;95:1167-72.

60. Verleden SE, Ruttens D, Vandermeulen E, et al. Elevated bronchoalveolar lavage eosinophilia correlates with poor outcome after lung transplantation. Transplantation 2014;97:83-9.

61. Saito T, Liu M, Binnie M, et al. Distinct expression patterns of alveolar "alarmins" in subtypes of chronic lung allograft dysfunction. Am J Transplant 2014;14:1425-32.

62. Vandermeulen E, Lammertyn E, Verleden SE, et al. Immunological diversity in phenotypes of chronic lung allograft dysfunction: a comprehensive immunohistochemical analysis. Transpl Int 2017;30:134-43.

63. Vandermeulen E, Verleden SE, Bellon H, et al. Humoral immunity in phenotypes of chronic lung allograft dysfunction: A broncho-alveolar lavage fluid analysis. Transpl Immunol 2016;38:27-32.

64. Levine DJ, Glanville AR, Aboyoun C, et al. Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2016;35:397-406.

65. Roux A, Bendib Le Lan I, Holifanjaniaina S, et al.

Antibody-Mediated Rejection in Lung Transplantation: Clinical Outcomes and Donor-Specific Antibody Characteristics. Am J Transplant 2016;16:1216-28.

66. Montero MA, Osadolor T, Khiroya R, et al. Restrictive allograft syndrome and idiopathic pleuroparenchymal fibroelastosis: do they really have the same histology? Histopathology 2017;70:1107-13.

67. Traxler D, Schweiger T, Schwarz S, et al. The Lymphatic Phenotype of Lung Allografts in Patients With Bronchiolitis Obliterans Syndrome and Restrictive Allograft Syndrome. Transplantation 2017;101:310-5.

68. Sato M, Hwang DM, Waddell TK, et al. Progression pattern of restrictive allograft syndrome after lung transplantation. J Heart Lung Transplant 2013;32:23-30.

69. Verleden SE, Todd JL, Sato M, et al. Impact of CLAD Phenotype on Survival After Lung Retransplantation: A Multicenter Study. Am J Transplant 2015;15:2223-30.

70. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet 2011;377:1760-9.

71. Vos R, Verleden SE, Ruttens D, et al. Pirfenidone: a potential new therapy for restrictive allograft syndrome? Am J Transplant 2013;13:3035-40.

72. Suhling H, Bollmann B, Gottlieb J. Nintedanib in restrictive chronic lung allograft dysfunction after lung transplantation. J Heart Lung Transplant 2016;35:939-40.

73. Kohno M, Perch M, Andersen E, et al. Treatment of intractable interstitial lung injury with alemtuzumab after lung transplantation. Transplant Proc 2011;43:1868-70.

74. Reams BD, Musselwhite LW, Zaas DW, et al. Alemtuzumab in the treatment of refractory acute rejection and bronchiolitis obliterans syndrome after human lung transplantation. Am J Transplant 2007;7:2802-8.

75. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. J Heart Lung Transplant 2010;29:973-80.

Prevention of chronic rejection after lung transplantation

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Abstract: Long-term survival after lung transplantation (LTx) is limited by chronic rejection (CR). Therapeutic strategies for CR have been largely unsuccessful, making prevention of CR an important and challenging therapeutic approach. In the current review, we will discuss current clinical evidence regarding prevention of CR after LTx.

Keywords: Chronic lung allograft dysfunction; lung transplantation (LTx); prevention; treatment

Introduction

During the last decades, short-term outcomes after lung transplantation (LTx) have improved due to advances in immunosuppressive regimens and better peri- and postoperative care. Despite significant improvement of early survival, however, long-term survival remains limited, with a median 5-year survival of approximately 54% and a median 10-year survival of 32% (1). Long-term survival after LTx is mainly hampered by the development of chronic rejection (CR) (1,2). CR in this review is defined as a sustained decrease in pulmonary function (forced expiratory volume in one second, FEV_1) of ≥20% from the average of the patient's two best post-transplant values ('baseline'), in the absence of other identifiable causes. CR affects 45% of all LTx recipients within 5 years following LTx, which is the highest prevalence in solid-organ transplantations, and accounts for the majority of late post-transplant mortality (3,4). There are at least two different clinical manifestations of CR: bronchiolitis obliterans syndrome (BOS)—an obstructive phenotype— and restrictive allograft syndrome (RAS) or restrictive CR— a restrictive phenotype. Median survival after BOS diagnosis is 3 to 5 years, whereas median survival after RAS diagnosis is limited to 0.5 to 1.5 years (5,6).

Since long-term survival after LTx is hampered by the development of CR and therapeutic strategies have been largely unsuccessful, prevention of CR is an important and challenging therapeutic approach (7-11). Current prophylactic practices mostly include an immunosuppressive induction therapy, a maintenance therapy with conventional triple drug immunosuppression (mostly methylprednisolone, a calcineurin inhibitor and a cytostatic agent) and prevention of risk factors for CR, including conventional infectious prophylaxis.

Despite the importance of preventive strategies, randomized trials are scarce. In the current paper, we will review clinical evidence aimed at prevention of CR after LTx (schematically summarized in *Figure 1*). Data derived from *in vitro* or animal studies are considered to be too premature and outside the scope of the current review.

Immunosuppression

Induction therapy

Induction therapy is an intense immunosuppressive therapy administered at the time of LTx with the aim of reducing early acute rejection (AR). AR has been shown to be the
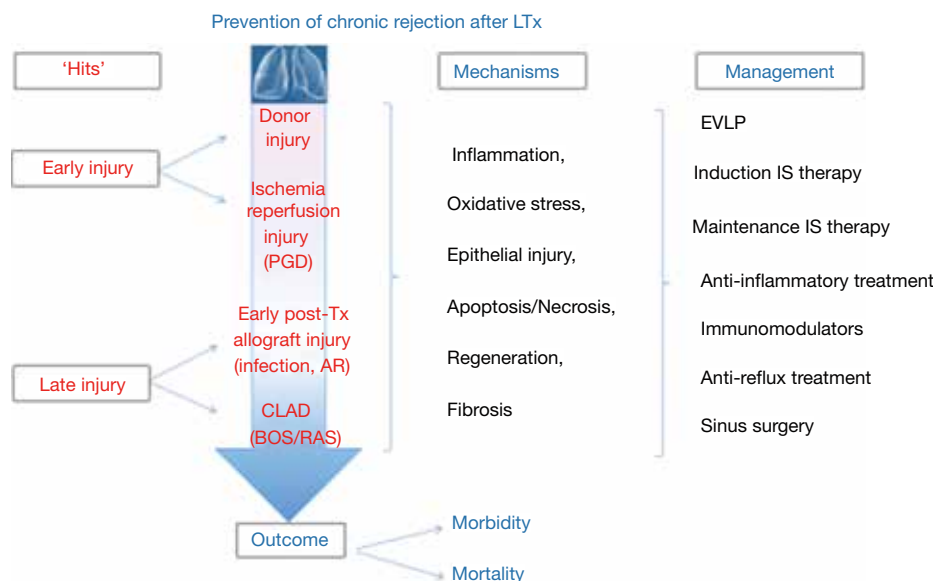
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Figure 1 Schematic presentation of the possible strategies for prevention of CR after LTx. PGD, primary graft dysfunction; AR, acute rejection; CLAD, chronic lung allograft dysfunction; BOS, bronchiolitis obliterans syndrome; RAS, restrictive allograft syndrome; EVLP, ex vivo lung perfusion; IS, immunosuppression; LTx, lung transplantation.

leading risk factor for CR. Induction therapy may reduce the incidence of early AR and hence, the subsequent development of CR (12). Also, induction therapy provides a longer timeframe to achieve therapeutic calcineurin inhibitor levels in the early post-operative period, allowing the kidneys to recover after surgery before experiencing toxic effects of CNI (9). Two categories of induction therapy are widely used: monoclonal or polyclonal lymphocyte-depleting agents and interleukin-2 receptor antagonists (IL2RA). According to the International Society for Heart and Lung Transplantation (ISHLT) registry, approximately 70% of LTx patients nowadays receive an induction treatment, most of them with IL2RA (1).

IL2RAs: daclizumab, basiliximab

Daclizumab and basiliximab are monoclonal agents directed against the interleukin-2 receptor of T-cells. Daclizumab and basiliximab saturate the alpha-subunit of this receptor, thereby preventing para-/autocrine activation and proliferation of T-cells by interleukin-2 (9,13). Evidence concerning these induction agents is conflicting. Multiple small retrospective and prospective trials demonstrated a decrease in AR episodes and prolonged CR-free and overall survival with daclizumab induction in comparison to antithymocyte globulin (ATG) induction or conventional immunosuppression with corticosteroids, a calcineurin

inhibitor and a cytostatic agent (14-16). In contrast however, other small retrospective trials demonstrated an increase in episodes of AR and CR incidence with daclizumab or basiliximab compared with ATG (17,18). In addition, a prospective trial comparing OKT3, ATG and daclizumab induction found no difference in episodes of AR, CR-free or overall survival between these three groups (19).

Lymphocyte-depleting agents: ATG, OKT3, alemtuzumab

ATG is a polyclonal lymphocyte-depleting agent that acts through complement mediated and antibody-related cell lysis and through opsonization and phagocytosis by macrophages (9,13). ATG was demonstrated in a RCT to be superior to conventional immunosuppression with corticosteroids, a calcineurin inhibitor and a cytostatic agent in preventing early AR (20). However, there were no differences in late post-transplant outcome, such as CR and survival (21).

OKT3 is a monoclonal lymphocyte-depleting agent that acts through binding of the T-cell receptor CD3 complex (9,13). Two prospective trials could not demonstrate a difference in AR, CR-free and overall survival after OKT3 induction compared to induction with ATG (19,22). Moreover, OKT3 was associated with more adverse effects, in particular with more bacterial infections (19).

Alemtuzumab is a monoclonal lymphocyte-depleting agent that acts through binding of CD52 on the cell surface of B and T cells, monocytes, macrophages and natural killer cells (9,13). In a study of Wehman et al. alemtuzumab induction with reduced immunosuppression showed a comparable rate of AR and overall survival in comparison to conventional immunosuppression with corticosteroids, a calcineurin inhibitor and a cytostatic agent (23). In addition, Shyu et al. could demonstrate an improved freedom from AR and lymphocytic bronchiolitis and a prolonged CR-free survival in LTx patients induced with alemtuzumab in comparison to LTx patients induced with ATG, daclizumab or conventional immunosuppression. Overall survival after induction with alemtuzumab was comparable to ATG induction and better than induction with daclizumab or conventional immunosuppression (24). However, alemtuzumab was only introduced the last decade, and evidence from randomized controlled trials is lacking.

A recent meta-analysis could not demonstrate any statistically significant differences between the use of T-cell antibody induction compared with no induction, or different types of T-cell antibodies discussed above regarding AR, CR-free and overall survival (25). Nevertheless, this meta-analysis was limited to the size and nature of the limited number of available studies.

Maintenance therapy

LTx patients usually receive a triple drug immunosuppression maintenance therapy consisting of corticosteroids, a cytostatic agent and a calcineurin inhibitor. Such a regimen provides more effective immunosuppression and minimizes side effects of drugs utilized by allowing lower target levels (1,9). According to the ISHLT, the most used combination therapy at 1 and 5 years post-LTx consists of tacrolimus-mycophenolate mofetil (MMF)-corticosteroids (1).

Corticosteroids

Corticosteroids attenuate inflammation by inducing neutrophil leukocytosis and a transient reduction in circulating eosinophils, monocytes, and lymphocytes. In contrast, antibody production is preserved, since B-cells are less affected by corticosteroids (26). Systemic corticosteroid use is widely considered an important component of immunosuppressive regimens after solid organ transplantation, despite numerous and frequent side-effects (1,27). Despite their widespread use in most immunosuppressive regimens, no randomized trials on

prevention of CR after LTx are available. Whether systemic corticosteroids can be safely stopped after LTx, without increasing the risk for subsequent development of CR, remains also unknown.

Airway inflammation is known to be suppressed by inhaled corticosteroids in other chronic inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) (28,29). Inhaled corticosteroids have been shown to reduce the total cell numbers in bronchoalveolar (BAL) fluid of LTx patients in a randomized placebo-controlled trial (30). In contrast with these findings, neither cell differentials, nor lung function were altered after adding 3 months inhaled corticosteroids to a systemic therapy of corticosteroids in LTx (31). These findings suggest that inhaled corticosteroids are ineffective for the prevention of CR after LTx, although more research is needed regarding adequate local delivery and timing of this therapy.

Cytostatic agents: azathioprine (AZA) and MMF

AZA and MMF are antimetabolites or antiproliferative agents which inhibit the purine and/or pyrimidine synthesis and thus block the de novo pathway of nucleotide synthesis in cells. In addition to this de novo pathway, a salvage pathway also provides nucleotide synthesis in most cells. As lymphocytes lack a salvage pathway, AZA and MMF specifically exert their antiproliferative effect on these cells (26,27).

MMF has proven to decrease the incidence of AR and to prolong overall and CR-free survival in renal and heart transplant recipients compared to AZA (32,33). LTx recipients treated with MMF also showed less episodes of AR and a slower decline in FEV_1 in comparison to AZA in non-randomized trials (34-36). Moreover, MMF significantly reduced graft loss due to CR in comparison to AZA in LTx (37). In contrast to these findings, two randomized trials could not demonstrate a difference in AR rates, CR-free and overall survival between MMF or AZA maintenance treatment (38,39). Therefore, despite the increasing use of MMF, there is limited evidence of superiority of MMF over AZA in LTx (9).

Calcineurin inhibitors: cyclosporine, tacrolimus

Cyclosporine forms a complex with cyclophilin, while tacrolimus binds to FK-binding protein or immunophilin in the cell cytoplasm of T-cells. These complexes both inhibit calcineurin from translocating to the nucleus, resulting in a lack of IL-2 mRNA transcription, which is needed for activation and proliferation of T lymphocytes. The

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limited amount of calcineurin in immune cells compared to other cell types, as well as the fact that calcineurin is critical for T-cell activation, accounts for the sensitivity of T-lymphocytes to cyclosporine and tacrolimus (9,40).

Tacrolimus has demonstrated superiority over cyclosporine in a limited number of randomized studies regarding CR incidence, CR-free survival, lymphocytic bronchiolitis and arterial hypertension (41-45), without an effect on AR or survival (41,43,45). On the other hand, tacrolimus may be associated with a higher incidence of post-transplant diabetes (44,46). Single center placebo-controlled trials could demonstrate an extended CR-free and overall survival when adding inhaled cyclosporine to the maintenance immunosuppression therapy after LTx (47-49). However, a subsequent multi-centre randomized controlled trial failed to demonstrate a difference in CR-free survival and overall survival (50).

Mammalian target of rapamycin (mTOR) inhibitors: everolimus, sirolimus

Everolimus and sirolimus block mTOR and inhibit growth factor-stimulated proliferation of lymphocytes and mesenchymal cells. According to the ISHLT registry, the use of mTOR inhibitors increases from approximately 8% 1 year after transplantation versus approximately 16% 5 years after LTx (1,9).

Two multicenter randomized trials could not show a difference in CR incidence, CR-free and overall survival between everolimus and AZA, or everolimus and *de novo* enteric-coated mycophenolate sodium as part of the triple immunosuppression regimen after LTx. Moreover, everolimus was associated with more severe adverse events (39,51). Similarly, Bhorade *et al.* could not demonstrate superiority of sirolimus in comparison to AZA regarding AR rate and incidence of CR (52). Sacher *et al.* on the other hand showed a lower incidence of CR and improved overall survival in patients treated with sirolimus compared to patients treated with MMF (53). However, sirolimus was also associated with significant adverse effects including venous thromboembolism and impaired bronchial anastomosis healing (52-54).

However, therapy with a mTOR inhibitor may provide an advantage in specific situations. Long-term calcineurin inhibitor use often leads to nephrotoxicity. Adding an mTOR to minimize calcineurin inhibitor exposure, may improve renal function without significant change in AR and FEV_1 (55,56). Additionally, mTOR inhibitors seem to be associated with a decreased incidence of cytomegalovirus

(CMV) infections in solid-organ transplant patients (52,57).

Immunomodulation

Neomacrolide antibiotics

Azithromycin and clarithromycin are neomacrolide antibiotics with a macrolactam ring. Both antibiotics are derived from erythromycin, an antibiotic with a macrocyclic lactone ring, which has been isolated from *Streptomyces* species (58).

Azithromycin

Azithromycin has both antimicrobial and immunomodulatory properties (59). Antimicrobial properties against gram-positive (e.g., *S. pneumoniae* and *S. aureus*), gram-negative (e.g., *H. influenzae* and *M. catarrhalis)* and atypical pathogens (e.g., *Chlamydia*, *Mycoplasma*, *Listeria*, *Pneumocystis* and *Legionella* spp.) are achieved by binding of the 50S ribosome subunit of bacteria, lowering protein synthesis. Antimicrobial activity against *P. Aeruginosa* is accomplished by reducing quorum sensing-dependent virulence factors, reducing alginate and biofilm formation, reducing protein synthesis and interaction with outer cellular membrane proteins (58). Immunomodulatory properties are achieved by influencing multiple cytokines and chemokines, and in this way lowering airway inflammation and airway remodeling. Exact cellular mechanisms are beyond the scope of this review and are summarized elsewhere (59).

Several studies have demonstrated azithromycin to improve FEV_1 by an average of 15% in 30–83% and overall survival in approximately 35–40% of LTx recipients diagnosed with CR (60-70). A randomized controlled trial of Corris *et al.* in established BOS demonstrated a mean difference in FEV_1 of 0.3 L in favor of azithromycin versus placebo (70). Especially patients with increased BAL lavage neutrophilia (>15–20%) are more successfully treated (67). In some of these azithromycin-treated patients, FEV_1 no longer meets the spirometric criteria for CR, which condition is denominated neutrophilic reversible allograft dysfunction (NRAD) or azithromycin responsive allograft dysfunction (ARAD) (67,71). NRAD/ARAD is typically characterized by neutrophilic airway inflammation, coarse crackles and increased sputum production and bronchiectasis, airway wall thickening, mucous plugging and centrilobular nodules on chest CT. Histology initially shows an inflammatory process, but may end up in fibrosis. Prognosis of NRAD/ARAD is good (71). Therefore,

current management guidelines recommend a trial of azithromycin for a least 3 months once CR is suspected (72).

Importantly, azithromycin is also effective as preventive therapy for CR. A randomized placebo-controlled trial of azithromycin demonstrated that prophylactic azithromycin reduces CR prevalence and improves CR-free survival. In this trial, CR prevalence is reduced with 32% after 2 years and with 23% after 7 years (73,74). Overall survival between the two groups is similar, most likely due to initiation of open-label azithromycin treatment in patients with established CR. Patients treated with azithromycin also demonstrated higher pulmonary function (FEV_1), better functional exercise capacity, lower airway neutrophilia and lower systemic C-reactive protein levels over time compared to those receiving placebo (73,74).

In patients with established NRAD/ARAD, multiple cytokines/chemokines and proteins are upregulated. One study shows that MMP-gelatinase is increased in BAL fluid of these patients. After 3 to 6 months of treatment with azithromycin, MMP-gelatinase was decreased compared with patients not treated with azithromycin, but still increased compared to stable patients. These findings suggest an ongoing matrix remodeling process, despite treatment with azithromycin, and thus possibly a higher risk to later development of CR. These findings suggest that a preventive approach with azithromycin may be preferred to a therapeutic approach (71).

Possible adverse events of azithromycin include nausea, vomiting, diarrhea or abdominal pain due to stimulation of gut motility. Cardiovascular events, particularly arrhythmias, mainly in case of concomitant use of other QT-prolonging medicines or underlying structural heart disease should be taken into account (75). However, the absolute risk of fatal cardiac arrhythmia is low and in fact comparable to that of alternative antibiotics (76). Azithromycin may be considered the safest of all macrolides since serum levels in healthy volunteers are >3,000 times lower than drug concentrations required for cardiomyocyte potassium channel (hERG/IKr)-blockade, action potential prolongation and QTc prolongation (22-24). Caution is nevertheless required for toxic accumulation of azithromycin in patients with impaired hepatic function, in case of concomitant use of other QT-prolonging drugs, or underlying structural heart disease. Another concern is the potential for emergence of bacterial resistance with long-term use of azithromycin, although this has not been show yet in microbiome studies in patients long-term treated with azithromycin (77,78).

Clarithromycin

Long-term treatment with clarithromycin in lung transplant recipients with established CR results in an improved FEV_1 in approximately one-third of patients in comparison to placebo. These results are comparable to long-term azithromycin therapy (79). On the other hand, Dhillon *et al.* could not demonstrate beneficial effects when using clarithromycin to prevent CR (80). The reason for this discrepancy with azithromycin is unclear. However, in contrast to clarithromycin, azithromycin does not affect calcineurin levels, making azithromycin the neomacrolide of preference for both prevention and treatment of CR post-LTx (81,82).

Montelukast (MLK)

MLK is a leukotriene-receptor antagonist (LTRA) with anti-inflammatory properties, which has particularly an effect on eosinophilic airway inflammation (83). A single center study in patients with pulmonary graft versus host syndrome disease after bone marrow transplantation, a disease showing similarities to CR after LTx, demonstrated an improvement in pulmonary function after treatment with MLK (84). Consecutively, a retrospective pilot study with MLK in patients with established CR, unresponsive to azithromycin, showed attenuation of the FEV_1 decline from 112±26 to 13±13 mL/month after 6 months treatment with MLK. In the control group, there was no significant change in the rate of FEV_1 decline (85). Unpublished data of our group demonstrate that in particular patients with BOS stage 1 unresponsive to azithromycin therapy may benefit from MLK treatment (Ruttens *et al.*, in revision).

Statins

Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase and by doing so reduce cholesterol. Besides this primary mechanism of action, statins also influence endothelial function and have immunomodulatory effects that are unrelated to their cholesterol-lowering function (86,87). Johnson *et al.* demonstrated that pulmonary function is better in LTx recipients post-operatively receiving statins in comparison to non-treated patients. Moreover, less and less severe episodes of AR and a better 6-year survival (91% versus 54%) were noted. Also a trend for a lower CR prevalence (0% versus 37%) was seen in statin-treated patients in comparison to placebo (88). Another study demonstrated an association between post-

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operative administration of statins and better CR-free and overall survival (89). These studies point to beneficial effect of preventive treatment with statins after LTx, yet the exact dose and duration of therapy is currently unknown. Also, he exact mechanism is unknown, but some of the beneficial effects can possibly be explained by reduction in primary graft dysfunction (PGD) grade 2 and grade 3 in patients taking statins prior to LTx (90). Since PGD is associated with an increased risk for CR one could argue that statins decrease CR development by reducing PGD (87,91). However, Johnson *et al.* demonstrated a trend towards a lower CR-free survival, despite that statins were started only 1 year after LTx (88). This suggests that statins, next to reducing PGD, may exert other effects which influence CR-free and overall survival.

Vitamin D

Vitamin D is a fat-soluble vitamin obtained by diet or through the skin that requires conversion in the liver or the kidneys to become metabolically active. Vitamin D was shown to have beneficial effects in asthma, COPD, and tuberculosis, demonstrating improved pulmonary function, reduced airway remodeling and a decrease in exacerbations (92,93). Since vitamin D has immunomodulatory functions, there may also be a possible beneficial effect of a preventive treatment with high doses of vitamin D after LTx (92). However, a randomized controlled trial could not demonstrate an improvement in CR prevalence and CR-free survival with vitamin D. Other secondary endpoints, including AR, were not different as well (94).

Extracorporeal photopheresis (ECP)

Intensified immunosuppression is obtained with ECP, in which leucocytes in the blood are isolated, incubated with 8-methoxypsoralen and subsequently exposed to ultraviolet-A light before re-entering the patient. This induces lymphocyte apoptosis, which likely causes an immunomodulatory effect via increase and activation of regulatory T-cell activation and thus can be considered as a type of T-cell vaccination (95).

Several studies demonstrated stabilization or improvement of FEV_1 and a better overall survival after addition of ECP to standard immunosuppressive therapy in LTx recipients diagnosed with CR (95-99). In particular LTx patients diagnosed with BOS with elevated BAL neutrophilia and slow decline in FEV_1 (<100 mL/month)

seem to benefit from ECP (95). LTx patients with recurrent episodes of AR were also found to have an overall superior survival after ECP treatment, making ECP possibly a promising treatment to prevent CR (100). Currently, early post-LTx ECP as pre-emptive strategy is being investigated (abstract of unpublished data: Jaksch *et al.* J Heart Lung Transplant 2014;32:803).

Prevention of risk factors

Ischemia-reperfusion injury

PGD due to ischemia-reperfusion injury, remains an important short and long-term complication after LTx (101). PGD occurs within the first 72 hours after LTx and is characterized by pulmonary edema with diffuse alveolar damage and infiltrates on chest CT. PGD clinically manifests itself as hypoxemia and is associated with CR-free and overall survival (102-105).

New preservation strategies of donor lungs revealed possibilities for attenuating PGD and increasing CR-free and overall survival. Improved preservation solutions, such as low potassium dextran and Celsior, have been associated with improved outcome (106,107). However, clinically ex vivo lung perfusion (EVLP) shows the greatest potential as *ex vivo* assessment of the lungs allows interventions to potentially improve lung quality by reducing inflammation and early immune activation. A leukocyte filter can decrease the load of inflammatory cells within the graft, while treatment with mesenchymal stem cells lead to a decrease in pulmonary inflammation (108,109). Tikkanen *et al.* could demonstrate an increased CR-free and overall survival when using EVLP in contrast to cold preservation of donor lungs, particularly in brain death donors (110). Long-term clinical results, including pulmonary function and CR, from a prospective, randomised, multi-center trial (INSPIRE) to compare lung allograft preservation by EVLP with standard cold storage are expected in 2017.

AR

Acute (perivascular) rejection (AR) is diagnosed based on the presence of perivascular and interstitial mononuclear cell infiltrates on transbronchial biopsy. The severity of AR is defined by the distribution and extension of these infiltrates and ranges from A0 (no rejection) to A4 (severe) (111). Episodes of minimal (A1), but especially mild and severe AR (A ≥2) are associated with higher incidence of CR

(112-115). The significance and treatment of minimal AR (grade A1) remains controversial. However, when grade A1 rejection is diagnosed, treatment with a short course of systemic steroids is recommended since the possible benefits of this therapy exceed the risks (12,72,112,116). Case-control studies with augmented immunosuppression in patients with non-minimal AR (grade ≥A1) after LTx are not available, since it is widely accepted that augmenting immunosuppression and a short course of systemic steroids is the appropriate treatment of non-minimal AR (1,72). However, indirect evidence suggests that augmented immunosuppression and a short course of systemic steroids may decrease the risk of subsequent CR in patients with non-minimal AR (113,117).

Lymphocytic bronchiolitis

Lymphocytic bronchiolitis represents acute airway inflammation and is characterized by a lymphocytic infiltration in the submucosa of bronchioles on histopathology with grades range from B0 (no rejection) to B2R (high grade rejection) (111). Lymphocytic bronchiolitis is an established risk factor for CR (88,90,91,94,95). Azithromycin decreases IL-17 driven neutrophilia in lymphocytic bronchiolitis and may as such reduces CR incidence (118). A short course of systemic steroids is advised as well, although convincing evidence is lacking (72). One study demonstrated a beneficial effect of inhaled steroids in LB, but evidence for preventive treatment with inhaled steroids is lacking (119).

Anti-human leucocyte antigen (HLA) antibodies

Donor specific antibodies (DSA), mostly anti-HLA antibodies, but also non-HLA antibodies, originate when the lung allograft is recognized by the recipient's immune system as non-self despite immunosuppressive treatment (120-126).

HLA antibodies have been associated with persistent, recurrent, high-grade AR and to lymphocytic bronchiolitis (125,126). Some 10% to 50% of LTx patients may develop DSA (mostly DQ) (121). Detection of *de novo* HLA DSA is linked with severity of CR and overall survival (121-124). Non-HLA antibodies to self-antigens (for example collagen V, K-α1 tubulin) are also known to be associated with an increased risk of CR and worse outcome after LTx (120-124). These findings suggest that (non-)HLA antibodies may have a pathogenic role in the development of CR and that clearing or preventing formation of these antibodies might reduce CR-prevalence.

In a study of Snyder *et al.*, an aggressive desensitization protocol with plasmapheresis, solumedrol, bortezomib and rituximab did not decrease HLA antibodies in sensitized LTx recipients, nor improve survival (127). In a prospective observational study of Hachem *et al.*, patients were started on preemptive antibody-directed therapy with rituximab and monthly intravenous immunoglobulins (IVIG) or monthly IVIG alone once they developed DSA. Patients who cleared their DSA had a better CR-free and overall survival compared to patients who failed to clear their DSA. Combined treatment with rituximab and IVIG proved to be superior compared to IVIG alone (128). Therefore, preemptive antibody-directed treatment may mitigate the risk of CR associated with DSA. However, evidence is scarce and placebo controlled trials are desperately needed.

Infections

Since the lung is one of the only organs in direct contact with the external environment, the lung is uniquely susceptible to microbial invasion, a part from the risk of donor-derived infections such as for instance by CMV. Therefore, infection of the lung allografts with micro-organisms is very frequent and multiple infections are known to be associated with the development of CR (129).

CMV

Since LTx donor and LTx recipient are most not matched for CMV status, reactivation of CMV after LTx is common. In the past, early survival after LTx was frequently hampered by CMV infections (130). Meanwhile, a preventive therapy with ganciclovir or valganciclovir has shown to reduce the cumulative incidence of CMV-related events and to ameliorate CR-free and overall survival in LTx patients (131-134). However, despite this improvement, CR-free and overall survival is still reduced in CMV-mismatched LTx recipients in comparison to matched patients despite ganciclovir/ganciclovir prophylaxis, suggesting that CMV prophylaxis is not optimized yet (135). Zamora *et al.* could show that ganciclovir/valganciclovir prophylaxis should be continued for at least 180 days after LTx to significantly reduce the incidence of CMV disease and hence CR-free and overall survival. However, optimal duration and timing of this preventive therapy remains unknown (136).

Multiple retrospective studies could demonstrate superiority of a combined preventive regimen consisting of CMV hyperimmune globulins (CMV IG) in addition

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to ganciclovir/valganciclovir in comparison to ganciclovir/valganciclovir alone in high risk patients influencing CR incidence and overall survival (135,137,138). Moreover, Solidoro *et al.* showed reduced AR and lymphocytic bronchiolitis, as well as a lower prevalence of Epstein-Barr virus (EBV) and combined CMV and EBV infections with a combined preventive therapy (139).

In the future, prospective randomized clinical trials are needed to evaluate efficacy, cost-effectiveness and timing of either prolonged ganciclovir/valganciclovir prophylaxis, or combined CMV prophylaxis on CR prevention.

Respiratory syncytial virus (RSV)

In LTx patients, RSV is one of the most common community-acquired respiratory virus infections. Lower respiratory tract infections by RSV are associated with the development of CR and a worse long-term survival (140-143). In the past, RSV has been treated off-label with ribavirin, corticosteroids, palivizumab or immunoglobulins. Nevertheless, none of these treatments was proven to prevent subsequent development of CR (144-146). However, recently a multicenter randomized placebo-controlled trial of Gottlieb *et al.* using inhaled ALN-RSV01 was able to demonstrate a lower incidence of new onset or progressive CR after RSV infection, compared to placebo. ALN-RSV01 is a siRNA targeting the RSV nucleocapsid messenger RNA, preventing formation of the nucleocapsid protein and thereby reducing viral replication. ALN-RSV01 may provide a novel preventive treatment approach for decreasing the incidence of RSV-related CR (147,148).

Pseudomonas spp.

Colonization or infection with *Pseudomonas* spp. after LTx is an established risk factor for the development of CR and is associated with worse CR-free and overall survival (129,149-151). *Pseudomonas* spp. induce direct tissue damage, immune-mediated injury and fibroblast proliferation, finally leading to complete obstruction of the airways (152). This process is orchestrated by intercellular quorum-sensing signaling molecules (QSM), which have an intrinsic immunomodulatory capacity resulting in suppressed T-cell proliferation, chemokine and cytokine release an neutrophil chemotaxis (149,153).

Azithromycin has immunomodulatory properties and inhibits the production of cytokines, proteases and quorum sensing molecules. Therefore, azithromycin may be an effective preventive therapy for CR in patients colonized with *Pseudomonas* after LTx (149). However, more prospective trials are needed. Aerosolized antipseudomonals (AAP) have demonstrated, in a single center retrospective trial, to protect LTx patients not suffering from cystic fibrosis (CF) against recurrent infections with *Pseudomonas* spp., without having an effect on CR-free and overall survival (154). AAP after LTx have also shown to improve maintenance of lower airway sterility concerning *Pseudomonas* spp. in CF and have possibly a beneficial effect on AR and CR occurrence or progression (155,156). However, randomized controlled trials with AAP as a preventive treatment are lacking.

Aspergillus spp.

Colonization and infection of the lungs with *Aspergillus* spp. have been identified as a distinct risk factor for CR (129,157). Since deposition of *Aspergillus* conidia in the small airways is involved in *Aspergillus*-driven development of CR, in particular small conidia *Aspergillus* spp. are at risk for the development of CR (158). In contradiction, Peghin *et al.* could not confirm the association between colonization and infection with *Aspergillus* spp. and subsequent development of CR (159).

Nebulized liposomal amphotericin B decreases the incidence of infection and colonization with *Aspergillus* spp. and therefore may prevent CR (159,160). Voriconazole prophylaxis may also be used as a preventive strategy for *Aspergillus* in LTx patients, but voriconazole is associated with liver enzyme abnormalities and the development of cutaneous squamous cell carcinoma (161,162). Yet, studies evaluating the efficacy of anti-*Aspergillus* prophylaxis are scarce and generally underpowered to detect a significant reduction in CR incidence after *Aspergillus* prophylaxis after LTx.

Gastroesophageal reflux

Gastroesophageal reflux disease (GERD) is highly prevalent in patients with end-stage lung diseases awaiting LTx (163). Moreover, LTx recipients are prone to develop GERD after surgery due to post-operative iatrogenic vagal innervation, impaired cough reflexes and mucociliary clearance, and reduced gastric motility induced by immunosuppressive drugs such as calcineurin inhibitors (164). Acid and non-acidic reflux are known non-alloimmune risk factors for the development of CR by causing repetitive epithelial injury and are associated with worse pulmonary function (165-168).

Pharmacological treatment of reflux consists of histamine-2 receptor blockers, proton pump inhibitors

(PPI) and prokinetic agents to support gastric motility (169). Evidence for medical treatment of GERD and gastric aspiration is scarce. A therapy with a PPI could not reduce pepsin as a surrogate marker of aspiration in BAL fluid of LTx patients (166). However, azithromycin was demonstrated to decrease reflux, proximal reflux episodes and esophageal acid exposure. Bile acid levels in BAL were significantly reduced as well (170).

Fundoplication surgery (e.g., Nissen or Toupet fundoplication) is a last surgical option to prevent gastric aspiration. In a study of 30 pediatric LTx recipients, reflux burden and fundoplication were not associated with CR-free and overall survival (171). However, other studies demonstrated a decrease in AR and an improvement or stabilization in CR-free and overall survival after anti-reflux surgery (169,172-178). These beneficial effects of fundoplication seems to be based on improving the immune environment in the lungs of LTx patients (179). Since only retrospective observational studies and case series are available, and thus more evidence is needed, surgery should only be performed in well-selected patients with high evidence for GERD.

Sinus surgery

Patients with CF frequently suffer from chronic rhinosinusitis. The sinuses of these patients can be considered as a reservoir for bacteria, which can spread to the lower respiratory tract, where they can cause allograft colonization and infection (180,181). The upper and lower airways of patients with CF are also more often colonized with *Pseudomonas* spp. than the airways of LTx recipients with other underlying diseases (182). Since *Pseudomonas* spp. are known to be associated with the development of CR, it is possible that chronic rhinosinusitis with *Pseudomonas* spp. plays a role in the development of CR in LTx recipients with CF (149).

Extensive sinus surgery, consisting of endoscopic fronto-spheno-ethmoidectomy combined with daily nasal rinsing with a saline solution, after LTx in CF patients may lead to a decrease of approximately 30% in concurrent colonization of the higher and lower airways with *Pseudomonas* spp. and other bacteria (183-185). A significant decrease in clinically relevant pulmonary infections has also been demonstrated (183). Whereas Holzmann *et al.* could only show a trend towards a lower incidence of CR after sinus surgery and daily nasal care after LTx in CF patients, Vital *et al.* could reveal a significant decrease of CR (183-185). Leung *et al.* could not prove an

effect on overall nor on CR-free survival, but the protocol of this study included pretransplant sinus surgery limited to the maxillary sinus and the ethmoid in CF patients and lacked a postoperative nasal care program (186).

Conclusions

Long-term survival after LTx is mainly limited by the development of CR. Since therapeutic strategies have been largely unsuccessful, prevention of CR is an important and challenging therapeutic approach. CR is a term covering different phenotypes including BOS and RAS. Since these different phenotypes have different clinical characteristics, different pathophysiological mechanisms and survival differences, different prevention and treatment strategies will be needed. Therefore, a personalized, tailored therapeutic regimen will probably be the most effective approach in these LTx patients. Despite the importance of preventive strategies, clear evidence is scarce and more randomized controlled trials are desperately needed.

Acknowledgements

Footnote

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References

1. Lund LH, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Heart Transplantation Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1158-69.

2. Verleden GM, Dupont LJ, Van Raemdonck DE, et al. Lung transplantation: a 15-year single-center experience.

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Van Herck et al. Prevention of CR after lung transplantation

Clin Transpl 2007:121-30.

3. Sayegh MH, Carpenter CB. Transplantation 50 years later--progress, challenges, and promises. N Engl J Med 2004;351:2761-6.

4. Opelz G, Döhler B, Ruhenstroth A, et al. The collaborative transplant study registry. Transplant Rev (Orlando) 2013;27:43-5.

5. Verleden GM, Vos R, Vanaudenaerde B, et al. Current views on chronic rejection after lung transplantation. Transpl Int 2015;28:1131-9.

6. Sato M, Waddell TK, Wagnetz U, et al. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. J Heart Lung Transplant 2011;30:735-42.

7. Belperio JA, Weigt SS, Fishbein MC, et al. Chronic lung allograft rejection: mechanisms and therapy. Proc Am Thorac Soc 2009;6:108-21.

8. Hopkins PM, McNeil K. Evidence for immunosuppression in lung transplantation. Curr Opin Organ Transplant 2008;13:477-83.

9. Snell GI, Westall GP, Paraskeva MA. Immunosuppression and allograft rejection following lung transplantation: evidence to date. Drugs 2013;73:1793-813.

10. Scott AI, Sharples LD, Stewart S. Bronchiolitis obliterans syndrome: risk factors and therapeutic strategies. Drugs 2005;65:761-71.

11. Vos R, Verleden SE, Verleden GM. Chronic lung allograft dysfunction: evolving practice. Curr Opin Organ Transplant 2015;20:483-91.

12. Sharples LD, McNeil K, Stewart S, et al. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. J Heart Lung Transplant 2002;21:271-81.

13. Knoop C, Haverich A, Fischer S. Immunosuppressive therapy after human lung transplantation. Eur Respir J 2004;23:159-71.

14. Ailawadi G, Smith PW, Oka T, et al. Effects of induction immunosuppression regimen on acute rejection, bronchiolitis obliterans, and survival after lung transplantation. J Thorac Cardiovasc Surg 2008;135:594-602.

15. Garrity ER Jr, Villanueva J, Bhorade SM, et al. Low rate of acute lung allograft rejection after the use of daclizumab, an interleukin 2 receptor antibody. Transplantation 2001;71:773-7.

16. Lischke R, Simonek J, Davidová R, et al. Induction therapy in lung transplantation: initial single-center experience comparing daclizumab and antithymocyte globulin. Transplant Proc 2007;39:205-12.

17. Hachem RR, Chakinala MM, Yusen RD, et al. A comparison of basiliximab and anti-thymocyte globulin as induction agents after lung transplantation. J Heart Lung Transplant 2005;24:1320-6.

18. Burton CM, Andersen CB, Jensen AS, et al. The incidence of acute cellular rejection after lung transplantation: a comparative study of anti-thymocyte globulin and daclizumab. J Heart Lung Transplant 2006;25:638-47.

19. Brock MV, Borja MC, Ferber L, et al. Induction therapy in lung transplantation: a prospective, controlled clinical trial comparing OKT3, anti-thymocyte globulin, and daclizumab. J Heart Lung Transplant 2001;20:1282-90.

20. Palmer SM, Miralles AP, Lawrence CM, et al. Rabbit antithymocyte globulin decreases acute rejection after lung transplantation: results of a randomized, prospective study. Chest 1999;116:127-33.

21. Hartwig MG, Snyder LD, Appel JZ 3rd, et al. Rabbit anti-thymocyte globulin induction therapy does not prolong survival after lung transplantation. J Heart Lung Transplant 2008;27:547-53.

22. Barlow CW, Moon MR, Green GR, et al. Rabbit antithymocyte globulin versus OKT3 induction therapy after heart-lung and lung transplantation: effect on survival, rejection, infection, and obliterative bronchiolitis. Transpl Int 2001;14:234-9.

23. Wehman B, Griffith BP, Balwan A, et al. Long-term outcomes following alemtuzumab induction in lung transplantation. Heart Surg Forum 2013;16:E252-6.

24. Shyu S, Dew MA, Pilewski JM, et al. Five-year outcomes with alemtuzumab induction after lung transplantation. J Heart Lung Transplant 2011;30:743-54.

25. Penninga L, Møller CH, Penninga EI, et al. Antibody induction therapy for lung transplant recipients. Cochrane Database Syst Rev 2013;(11):CD008927.

26. van Sandwijk MS, Bemelman FJ, Ten Berge IJ. Immunosuppressive drugs after solid organ transplantation. Neth J Med 2013;71:281-9.

27. Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 2005;56:23-46.

28. Vogelmeier CF, Criner GJ, Martinez FJ, et al. Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. Respirology 2017;22:575-601.

29. (*NEW) 2017 GINA Report: Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma - GINA. Available online: http://ginasthma.org/2017-

gina-report-global-strategy-for-asthma-management-and-prevention/

30. Whitford H, Orsida B, Kotsimbos T, et al. Bronchoalveolar lavage cellular profiles in lung transplantation: the effect of inhaled corticosteroids. Ann Transplant 2000;5:31-7.

31. Whitford H, Walters EH, Levvey B, et al. Addition of inhaled corticosteroids to systemic immunosuppression after lung transplantation: a double-blind, placebo-controlled trial. Transplantation 2002;73:1793-9.

32. Halloran P, Mathew T, Tomlanovich S, et al. Mycophenolate mofetil in renal allograft recipients: a pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. The International Mycophenolate Mofetil Renal Transplant Study Groups. Transplantation 1997;63:39-47.

33. Kobashigawa J, Miller L, Renlund D, et al. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate Mofetil Investigators. Transplantation 1998;66:507-15.

34. Ross DJ, Waters PF, Levine M, et al. Mycophenolate mofetil versus azathioprine immunosuppressive regimens after lung transplantation: preliminary experience. J Heart Lung Transplant 1998;17:768-74.

35. Zuckermann A, Klepetko W, Birsan T, et al. Comparison between mycophenolate mofetil- and azathioprine-based immunosuppressions in clinical lung transplantation. J Heart Lung Transplant 1999;18:432-40.

36. O'Hair DP, Cantu E, McGregor C, et al. Preliminary experience with mycophenolate mofetil used after lung transplantation. J Heart Lung Transplant 1998;17:864-8.

37. Speich R, Schneider S, Hofer M, et al. Mycophenolate mofetil reduces alveolar inflammation, acute rejection and graft loss due to bronchiolitis obliterans syndrome after lung transplantation. Pulm Pharmacol Ther 2010;23:445-9.

38. Palmer SM, Baz MA, Sanders L, et al. Results of a randomized, prospective, multicenter trial of mycophenolate mofetil versus azathioprine in the prevention of acute lung allograft rejection. Transplantation 2001;71:1772-6.

39. Glanville AR, Aboyoun C, Klepetko W, et al. Three-year results of an investigator-driven multicenter, international, randomized open-label de novo trial to prevent BOS after lung transplantation. J Heart Lung Transplant 2015;34:16-25.

40. Enderby C, Keller CA. An overview of immunosuppression in solid organ transplantation. Am J Manag Care 2015;21:s12-23.

41. Keenan RJ, Konishi H, Kawai A, et al. Clinical trial of tacrolimus versus cyclosporine in lung transplantation. Ann Thorac Surg 1995;60:580-4; discussion 584-5.

42. Treede H, Klepetko W, Reichenspurner H, et al. Tacrolimus versus cyclosporine after lung transplantation: a prospective, open, randomized two-center trial comparing two different immunosuppressive protocols. J Heart Lung Transplant 2001;20:511-7.

43. Treede H, Glanville AR, Klepetko W, et al. Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome: results of a prospective, randomized international trial in lung transplantation. J Heart Lung Transplant 2012;31:797-804.

44. Zuckermann A, Reichenspurner H, Birsan T, et al. Cyclosporine A versus tacrolimus in combination with mycophenolate mofetil and steroids as primary immunosuppression after lung transplantation: one-year results of a 2-center prospective randomized trial. J Thorac Cardiovasc Surg 2003;125:891-900.

45. Penninga L, Penninga EI, Møller CH, et al. Tacrolimus versus cyclosporin as primary immunosuppression for lung transplant recipients. Cochrane Database Syst Rev 2013;(5):CD008817.

46. Hachem RR, Yusen RD, Chakinala MM, et al. A randomized controlled trial of tacrolimus versus cyclosporine after lung transplantation. J Heart Lung Transplant 2007;26:1012-8.

47. Iacono AT, Johnson BA, Grgurich WF, et al. A randomized trial of inhaled cyclosporine in lung-transplant recipients. N Engl J Med 2006;354:141-50.

48. Corcoran TE, Smaldone GC, Dauber JH, et al. Preservation of post-transplant lung function with aerosol cyclosporin. Eur Respir J 2004;23:378-83.

49. DeCamp MM Jr. Inhaled cyclosporine--a breath of fresh air? N Engl J Med 2006;354:191-3.

50. Johnson BA, Zamora MR, Budev MM, et al. 172 Cyclosporine Inhalation Solution Does Not Improve Bronchiolitis Obliterans Syndrome-Free Survival Following Lung Transplant: Results from the CYCLIST Trial. J Heart Lung Transplant 2012;31:S66.

51. Snell GI, Valentine VG, Vitulo P, et al. Everolimus versus azathioprine in maintenance lung transplant recipients: an international, randomized, double-blind clinical trial. Am J Transplant 2006;6:169-77.

52. Bhorade S, Ahya VN, Baz MA, et al. Comparison of sirolimus with azathioprine in a tacrolimus-based immunosuppressive regimen in lung transplantation. Am J

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Van Herck et al. Prevention of CR after lung transplantation

Respir Crit Care Med 2011;183:379-87.

53. Sacher VY, Fertel D, Srivastava K, et al. Effects of prophylactic use of sirolimus on bronchiolitis obliterans syndrome development in lung transplant recipients. Ann Thorac Surg 2014;97:268-74.

54. King-Biggs MB, Dunitz JM, Park SJ, et al. Airway anastomotic dehiscence associated with use of sirolimus immediately after lung transplantation. Transplantation 2003;75:1437-43.

55. Gullestad L, Eiskjaer H, Gustafsson F, et al. Long-term outcomes of thoracic transplant recipients following conversion to everolimus with reduced calcineurin inhibitor in a multicenter, open-label, randomized trial. Transpl Int 2016;29:819-29.

56. Shitrit D, Rahamimov R, Gidon S, et al. Use of sirolimus and low-dose calcineurin inhibitor in lung transplant recipients with renal impairment: results of a controlled pilot study. Kidney Int 2005;67:1471-5.

57. Ghassemieh B, Ahya VN, Baz MA, et al. Decreased incidence of cytomegalovirus infection with sirolimus in a post hoc randomized, multicenter study in lung transplantation. J Heart Lung Transplant 2013;32:701-6.

58. Retsema J, Fu W. Macrolides: structures and microbial targets. Int J Antimicrob Agents 2001;18 Suppl 1:S3-10.

59. Vos R, Vanaudenaerde BM, Verleden SE, et al. Anti-inflammatory and immunomodulatory properties of azithromycin involved in treatment and prevention of chronic lung allograft rejection. Transplantation 2012;94:101-9.

60. Gerhardt SG, McDyer JF, Girgis RE, et al. Maintenance azithromycin therapy for bronchiolitis obliterans syndrome: results of a pilot study. Am J Respir Crit Care Med 2003;168:121-5.

61. Verleden GM, Dupont LJ. Azithromycin therapy for patients with bronchiolitis obliterans syndrome after lung transplantation. Transplantation 2004;77:1465-7.

62. Yates B, Murphy DM, Forrest IA, et al. Azithromycin reverses airflow obstruction in established bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2005;172:772-5.

63. Shitrit D, Bendayan D, Gidon S, et al. Long-term azithromycin use for treatment of bronchiolitis obliterans syndrome in lung transplant recipients. J Heart Lung Transplant 2005;24:1440-3.

64. Porhownik NR, Batobara W, Kepron W, et al. Effect of maintenance azithromycin on established bronchiolitis obliterans syndrome in lung transplant patients. Can Respir J 2008;15:199-202.

65. Gottlieb J, Szangolies J, Koehnlein T, et al. Long-term azithromycin for bronchiolitis obliterans syndrome after lung transplantation. Transplantation 2008;85:36-41.

66. Verleden GM, Vanaudenaerde BM, Dupont LJ, et al. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2006;174:566-70.

67. Vanaudenaerde BM, Meyts I, Vos R, et al. A dichotomy in bronchiolitis obliterans syndrome after lung transplantation revealed by azithromycin therapy. Eur Respir J 2008;32:832-43.

68. Vos R, Vanaudenaerde BM, Ottevaere A, et al. Long-term azithromycin therapy for bronchiolitis obliterans syndrome: divide and conquer? J Heart Lung Transplant 2010;29:1358-68.

69. Jain R, Hachem RR, Morrell MR, et al. Azithromycin is associated with increased survival in lung transplant recipients with bronchiolitis obliterans syndrome. J Heart Lung Transplant 2010;29:531-7.

70. Corris PA, Ryan VA, Small T, et al. A randomised controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome (BOS) post lung transplantation. Thorax 2015;70:442-50.

71. Verleden SE, Vandermeulen E, Ruttens D, et al. Neutrophilic reversible allograft dysfunction (NRAD) and restrictive allograft syndrome (RAS). Semin Respir Crit Care Med 2013;34:352-60.

72. Meyer KC, Raghu G, Verleden GM, et al. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J 2014;44:1479-503.

73. Vos R, Vanaudenaerde BM, Verleden SE, et al. A randomised controlled trial of azithromycin to prevent chronic rejection after lung transplantation. Eur Respir J 2011;37:164-72.

74. Ruttens D, Verleden SE, Vandermeulen E, et al. Prophylactic Azithromycin Therapy After Lung Transplantation: Post hoc Analysis of a Randomized Controlled Trial. Am J Transplant 2016;16:254-61.

75. Li H, Liu DH, Chen LL, et al. Meta-analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases. Antimicrob Agents Chemother 2014;58:511-7.

76. Svanström H, Pasternak B, Hviid A. Use of azithromycin and death from cardiovascular causes. N Engl J Med 2013;368:1704-12.

77. Albert RK, Connett J, Bailey WC, et al. Azithromycin for prevention of exacerbations of COPD. N Engl J Med

2011;365:689-98.

78. Lopes Dos Santos Santiago G, Brusselle G, Dauwe K, et al. Influence of chronic azithromycin treatment on the composition of the oropharyngeal microbial community in patients with severe asthma. BMC Microbiol 2017;17:109.

79. Benden C, Boehler A. Long-term clarithromycin therapy in the management of lung transplant recipients. Transplantation 2009;87:1538-40.

80. Dhillon GS, Valentine VG, Levitt J, et al. Clarithromycin for prevention of bronchiolitis obliterans syndrome in lung allograft recipients. Clin Transplant 2012;26:105-10.

81. Ibrahim RB, Abella EM, Chandrasekar PH. Tacrolimus-clarithromycin interaction in a patient receiving bone marrow transplantation. Ann Pharmacother 2002;36:1971-2.

82. Kunicki PK, Sobieszczańska-Małek M. Pharmacokinetic interaction between tacrolimus and clarithromycin in a heart transplant patient. Ther Drug Monit 2005;27:107-8.

83. Ramsay CF, Sullivan P, Gizycki M, et al. Montelukast and bronchial inflammation in asthma: a randomised, double-blind placebo-controlled trial. Respir Med 2009;103:995-1003.

84. Or R, Gesundheit B, Resnick I, et al. Sparing effect by montelukast treatment for chronic graft versus host disease: a pilot study. Transplantation 2007;83:577-81.

85. Verleden GM, Verleden SE, Vos R, et al. Montelukast for bronchiolitis obliterans syndrome after lung transplantation: a pilot study. Transpl Int 2011;24:651-6.

86. Mach F. Statins as novel immunomodulators: from cell to potential clinical benefit. Thromb Haemost 2003;90:607-10.

87. Verleden GM, Vos R. Statins in lung transplantation: A treatment option for every patient? J Heart Lung Transplant 2017;36:936-7.

88. Johnson BA, Iacono AT, Zeevi A, et al. Statin use is associated with improved function and survival of lung allografts. Am J Respir Crit Care Med 2003;167:1271-8.

89. Li Y, Gottlieb J, Ma D, et al. Graft-protective effects of the HMG-CoA reductase inhibitor pravastatin after lung transplantation--a propensity score analysis with 23 years of follow-up. Transplantation 2011;92:486-92.

90. Raphael J, Collins SR, Wang XQ, et al. Perioperative statin use is associated with decreased incidence of primary graft dysfunction after lung transplantation. J Heart Lung Transplant 2017;36:948-56.

91. McAuley DF, Laffey JG, O'Kane CM, et al. Simvastatin in the acute respiratory distress syndrome. N Engl J Med 2014;371:1695-703.

92. Solidoro P, Bellocchia M, Facchini F. The immunobiological and clinical role of vitamin D in obstructive lung diseases. Minerva Med 2016;107:12-9.

93. Janssens W, Bouillon R, Claes B, et al. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax 2010;65:215-20.

94. Vos R, Ruttens D, Verleden SE, et al. High-dose vitamin D after lung transplantation: A randomized trial. J Heart Lung Transplant 2017;36:897-905.

95. Greer M, Dierich M, De Wall C, et al. Phenotyping established chronic lung allograft dysfunction predicts extracorporeal photopheresis response in lung transplant patients. Am J Transplant 2013;13:911-8.

96. Del Fante C, Scudeller L, Oggionni T, et al. Long-Term Off-Line Extracorporeal Photochemotherapy in Patients with Chronic Lung Allograft Rejection Not Responsive to Conventional Treatment: A 10-Year Single-Centre Analysis. Respiration 2015;90:118-28.

97. Jaksch P, Scheed A, Keplinger M, et al. A prospective interventional study on the use of extracorporeal photopheresis in patients with bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant 2012;31:950-7.

98. Morrell MR, Despotis GJ, Lublin DM, et al. The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant 2010;29:424-31.

99. Benden C, Speich R, Hofbauer GF, et al. Extracorporeal photopheresis after lung transplantation: a 10-year single-center experience. Transplantation 2008;86:1625-7.

100. Isenring B, Robinson C, Buergi U, et al. Lung transplant recipients on long-term extracorporeal photopheresis. Clin Transplant 2017;31.

101. Christie JD, Carby M, Bag R, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2005;24:1454-9.

102. Meyers BF, de la Morena M, Sweet SC, et al. Primary graft dysfunction and other selected complications of lung transplantation: A single-center experience of 983 patients. J Thorac Cardiovasc Surg 2005;129:1421-9.

103. Fiser SM, Tribble CG, Long SM, et al. Ischemia-reperfusion injury after lung transplantation increases risk of late bronchiolitis obliterans syndrome. Ann Thorac Surg 2002;73:1041-7; discussion 1047-8.

104. Christie JD, Kotloff RM, Ahya VN, et al. The

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Van Herck et al. Prevention of CR after lung transplantation

effect of primary graft dysfunction on survival after lung transplantation. Am J Respir Crit Care Med 2005;171:1312-6.

105. Suzuki Y, Cantu E, Christie JD. Primary graft dysfunction. Semin Respir Crit Care Med 2013;34:305-19.

106. Gohrbandt B, Simon AR, Warnecke G, et al. Lung Preservation With Perfadex or Celsior in Clinical Transplantation: A Retrospective Single-Center Analysis of Outcomes. Transplantation 2015;99:1933-9.

107. Latchana N, Peck JR, Whitson B, et al. Preservation solutions for cardiac and pulmonary donor grafts: a review of the current literature. J Thorac Dis 2014;6:1143-9.

108. Mohamed MS. Could Ex Vivo Lung Perfusion Be a Platform to Decrease the Incidence of Chronic Lung Allograft Dysfunction? Arch Med Res 2015;46:240-3.

109. Martens A, Ordies S, Vanaudenaerde BM, et al. Immunoregulatory effects of multipotent adult progenitor cells in a porcine ex vivo lung perfusion model. Stem Cell Res Ther 2017;8:159.

110. Tikkanen JM, Cypel M, Machuca TN, et al. Functional outcomes and quality of life after normothermic ex vivo lung perfusion lung transplantation. J Heart Lung Transplant 2015;34:547-56.

111. Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant 2007;26:1229-42.

112. Bando K, Paradis IL, Similo S, et al. Obliterative bronchiolitis after lung and heart-lung transplantation. An analysis of risk factors and management. J Thorac Cardiovasc Surg 1995;110:4-13; discussion 13-4.

113. Husain AN, Siddiqui MT, Holmes EW, et al. Analysis of risk factors for the development of bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 1999;159:829-33.

114. Girgis RE, Tu I, Berry GJ, et al. Risk factors for the development of obliterative bronchiolitis after lung transplantation. J Heart Lung Transplant 1996;15:1200-8.

115. Glanville AR, Aboyoun CL, Havryk A, et al. Severity of lymphocytic bronchiolitis predicts long-term outcome after lung transplantation. Am J Respir Crit Care Med 2008;177:1033-40.

116. Hopkins PM, Aboyoun CL, Chhajed PN, et al. Association of minimal rejection in lung transplant recipients with obliterative bronchiolitis. Am J Respir Crit Care Med 2004;170:1022-6.

117. Guilinger RA, Paradis IL, Dauber JH, et al. The importance of bronchoscopy with transbronchial biopsy and bronchoalveolar lavage in the management of

lung transplant recipients. Am J Respir Crit Care Med 1995;152:2037-43.

118. Vandermeulen E, Verleden SE, Ruttens D, et al. BAL neutrophilia in azithromycin-treated lung transplant recipients: Clinical significance. Transpl Immunol 2015;33:37-44.

119. De Soyza A, Fisher AJ, Small T, et al. Inhaled corticosteroids and the treatment of lymphocytic bronchiolitis following lung transplantation. Am J Respir Crit Care Med 2001;164:1209-12.

120. Hachem RR, Tiriveedhi V, Patterson GA, et al. Antibodies to K-α 1 tubulin and collagen V are associated with chronic rejection after lung transplantation. Am J Transplant 2012;12:2164-71.

121. Tikkanen JM, Singer LG, Kim SJ, et al. De Novo DQ Donor-Specific Antibodies Are Associated with Chronic Lung Allograft Dysfunction after Lung Transplantation. Am J Respir Crit Care Med 2016;194:596-606.

122. Morrell MR, Pilewski JM, Gries CJ, et al. De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. J Heart Lung Transplant 2014;33:1288-94.

123. Snyder LD, Wang Z, Chen DF, et al. Implications for human leukocyte antigen antibodies after lung transplantation: a 10-year experience in 441 patients. Chest 2013;144:226-33.

124. Safavi S, Robinson DR, Soresi S, et al. De novo donor HLA-specific antibodies predict development of bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant 2014;33:1273-81.

125. Girnita AL, McCurry KR, Iacono AT, et al. HLA-specific antibodies are associated with high-grade and persistent-recurrent lung allograft acute rejection. J Heart Lung Transplant 2004;23:1135-41.

126. Girnita AL, Duquesnoy R, Yousem SA, et al. HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. Am J Transplant 2005;5:131-8.

127. Snyder LD, Gray AL, Reynolds JM, et al. Antibody desensitization therapy in highly sensitized lung transplant candidates. Am J Transplant 2014;14:849-56.

128. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. J Heart Lung Transplant 2010;29:973-80.

129. Martin-Gandul C, Mueller NJ, Pascual M, et al. The

Impact of Infection on Chronic Allograft Dysfunction and Allograft Survival After Solid Organ Transplantation. Am J Transplant 2015;15:3024-40.

130. Duncan AJ, Dummer JS, Paradis IL, et al. Cytomegalovirus infection and survival in lung transplant recipients. J Heart Lung Transplant 1991;10:638-44; discussion 645-6.

131. Speich R, Thurnheer R, Gaspert A, et al. Efficacy and cost effectiveness of oral ganciclovir in the prevention of cytomegalovirus disease after lung transplantation. Transplantation 1999;67:315-20.

132. Soghikian MV, Valentine VG, Berry GJ, et al. Impact of ganciclovir prophylaxis on heart-lung and lung transplant recipients. J Heart Lung Transplant 1996;15:881-7.

133. Palmer SM, Grinnan DC, Diane Reams B, et al. Delay of CMV infection in high-risk CMV mismatch lung transplant recipients due to prophylaxis with oral ganciclovir. Clin Transplant 2004;18:179-85.

134. Chmiel C, Speich R, Hofer M, et al. Ganciclovir/valganciclovir prophylaxis decreases cytomegalovirus-related events and bronchiolitis obliterans syndrome after lung transplantation. Clin Infect Dis 2008;46:831-9.

135. Ruttmann E, Geltner C, Bucher B, et al. Combined CMV prophylaxis improves outcome and reduces the risk for bronchiolitis obliterans syndrome (BOS) after lung transplantation. Transplantation 2006;81:1415-20.

136. Zamora MR, Nicolls MR, Hodges TN, et al. Following universal prophylaxis with intravenous ganciclovir and cytomegalovirus immune globulin, valganciclovir is safe and effective for prevention of CMV infection following lung transplantation. Am J Transplant 2004;4:1635-42.

137. Valantine HA, Luikart H, Doyle R, et al. Impact of cytomegalovirus hyperimmune globulin on outcome after cardiothoracic transplantation: a comparative study of combined prophylaxis with CMV hyperimmune globulin plus ganciclovir versus ganciclovir alone. Transplantation 2001;72:1647-52.

138. Weill D, Lock BJ, Wewers DL, et al. Combination prophylaxis with ganciclovir and cytomegalovirus (CMV) immune globulin after lung transplantation: effective CMV prevention following daclizumab induction. Am J Transplant 2003;3:492-6.

139. Solidoro P, Libertucci D, Delsedime L, et al. Combined cytomegalovirus prophylaxis in lung transplantation: effects on acute rejection, lymphocytic bronchitis/bronchiolitis, and herpesvirus infections. Transplant Proc 2008;40:2013-4.

140. Glezen WP, Greenberg SB, Atmar RL, et al. Impact of respiratory virus infections on persons with chronic underlying conditions. JAMA 2000;283:499-505.

141. Khalifah AP, Hachem RR, Chakinala MM, et al. Respiratory viral infections are a distinct risk for bronchiolitis obliterans syndrome and death. Am J Respir Crit Care Med 2004;170:181-7.

142. Kumar D, Erdman D, Keshavjee S, et al. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after lung transplant. Am J Transplant 2005;5:2031-6.

143. Billings JL, Hertz MI, Savik K, et al. Respiratory viruses and chronic rejection in lung transplant recipients. J Heart Lung Transplant 2002;21:559-66.

144. Pelaez A, Lyon GM, Force SD, et al. Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. J Heart Lung Transplant 2009;28:67-71.

145. Danziger-Isakov LA, Arslan D, Sweet S, et al. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the International Pediatric Lung Transplant Collaborative. Pediatr Transplant 2012;16:638-44.

146. Liu V, Dhillon GS, Weill D. A multi-drug regimen for respiratory syncytial virus and parainfluenza virus infections in adult lung and heart-lung transplant recipients. Transpl Infect Dis 2010;12:38-44.

147. Gottlieb J, Zamora MR, Hodges T, et al. ALN-RSV01 for prevention of bronchiolitis obliterans syndrome after respiratory syncytial virus infection in lung transplant recipients. J Heart Lung Transplant 2016;35:213-21.

148. Zamora MR, Budev M, Rolfe M, et al. RNA interference therapy in lung transplant patients infected with respiratory syncytial virus. Am J Respir Crit Care Med 2011;183:531-8.

149. Vos R, Vanaudenaerde BM, Geudens N, et al. Pseudomonal airway colonisation: risk factor for bronchiolitis obliterans syndrome after lung transplantation? Eur Respir J 2008;31:1037-45.

150. Botha P, Archer L, Anderson RL, et al. Pseudomonas aeruginosa colonization of the allograft after lung transplantation and the risk of bronchiolitis obliterans syndrome. Transplantation 2008;85:771-4.

151. Gottlieb J, Mattner F, Weissbrodt H, et al. Impact of graft colonization with gram-negative bacteria after lung transplantation on the development of bronchiolitis obliterans syndrome in recipients with cystic fibrosis. Respir Med 2009;103:743-9.

152. Borthwick LA, Suwara MI, Carnell SC, et al. Pseudomonas aeruginosa Induced Airway Epithelial Injury Drives

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Van Herck et al. Prevention of CR after lung transplantation

Fibroblast Activation: A Mechanism in Chronic Lung Allograft Dysfunction. Am J Transplant 2016;16:1751-65.

153. Van Delden C, Iglewski BH. Cell-to-cell signaling and Pseudomonas aeruginosa infections. Emerg Infect Dis 1998;4:551-60.

154. Moore CA, Pilewski JM, Venkataramanan R, et al. Effect of aerosolized antipseudomonals on Pseudomonas positivity and bronchiolitis obliterans syndrome after lung transplantation. Transpl Infect Dis 2017;19.

155. Konstan MW, Geller DE, Minić P, et al. Tobramycin inhalation powder for P. aeruginosa infection in cystic fibrosis: the EVOLVE trial. Pediatr Pulmonol 2011;46:230-8.

156. Suhling H, Rademacher J, Greer M, et al. Inhaled colistin following lung transplantation in colonised cystic fibrosis patients. Eur Respir J 2013;42:542-4.

157. Weigt SS, Elashoff RM, Huang C, et al. Aspergillus colonization of the lung allograft is a risk factor for bronchiolitis obliterans syndrome. Am J Transplant 2009;9:1903-11.

158. Weigt SS, Copeland CA, Derhovanessian A, et al. Colonization with small conidia Aspergillus species is associated with bronchiolitis obliterans syndrome: a two-center validation study. Am J Transplant 2013;13:919-27.

159. Peghin M, Monforte V, Martin-Gomez MT, et al. 10 years of prophylaxis with nebulized liposomal amphotericin B and the changing epidemiology of Aspergillus spp. infection in lung transplantation. Transpl Int 2016;29:51-62.

160. Borro JM, Solé A, de la Torre M, et al. Efficiency and safety of inhaled amphotericin B lipid complex (Abelcet) in the prophylaxis of invasive fungal infections following lung transplantation. Transplant Proc 2008;40:3090-3.

161. Husain S, Paterson DL, Studer S, et al. Voriconazole prophylaxis in lung transplant recipients. Am J Transplant 2006;6:3008-16.

162. Kolaitis NA, Duffy E, Zhang A, et al. Voriconazole increases the risk for cutaneous squamous cell carcinoma after lung transplantation. Transpl Int 2017;30:41-8.

163. D'Ovidio F, Singer LG, Hadjiliadis D, et al. Prevalence of gastroesophageal reflux in end-stage lung disease candidates for lung transplant. Ann Thorac Surg 2005;80:1254-60.

164. Mohammed A, Neujahr DC. Gastroesophageal reflux disease and graft failure after lung transplantation. Transplant Rev (Orlando) 2010;24:99-103.

165. Hadjiliadis D, Duane Davis R, Steele MP, et al. Gastroesophageal reflux disease in lung transplant recipients. Clin Transplant 2003;17:363-8.

166. Blondeau K, Mertens V, Vanaudenaerde BA, et al. Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. Eur Respir J 2008;31:707-13.

167. Palmer SM, Miralles AP, Howell DN, et al. Gastroesophageal reflux as a reversible cause of allograft dysfunction after lung transplantation. Chest 2000;118:1214-7.

168. Young LR, Hadjiliadis D, Davis RD, et al. Lung transplantation exacerbates gastroesophageal reflux disease. Chest 2003;124:1689-93.

169. Hathorn KE, Chan WW, Lo WK. Role of gastroesophageal reflux disease in lung transplantation. World J Transplant 2017;7:103-16.

170. Mertens V, Blondeau K, Pauwels A, et al. Azithromycin reduces gastroesophageal reflux and aspiration in lung transplant recipients. Dig Dis Sci 2009;54:972-9.

171. Jamie Dy F, Freiberger D, Liu E, et al. Impact of gastroesophageal reflux and delayed gastric emptying on pediatric lung transplant outcomes. J Heart Lung Transplant 2017;36:854-61.

172. Davis RD Jr, Lau CL, Eubanks S, et al. Improved lung allograft function after fundoplication in patients with gastroesophageal reflux disease undergoing lung transplantation. J Thorac Cardiovasc Surg 2003;125:533-42.

173. Cantu E 3rd, Appel JZ 3rd, Hartwig MG, et al. J. Maxwell Chamberlain Memorial Paper. Early fundoplication prevents chronic allograft dysfunction in patients with gastroesophageal reflux disease. Ann Thorac Surg 2004;78:1142-51; discussion 1142-51.

174. Lau CL, Palmer SM, Howell DN, et al. Laparoscopic antireflux surgery in the lung transplant population. Surg Endosc 2002;16:1674-8.

175. Burton PR, Button B, Brown W, et al. Medium-term outcome of fundoplication after lung transplantation. Dis Esophagus 2009;22:642-8.

176. Hartwig MG, Anderson DJ, Onaitis MW, et al. Fundoplication after lung transplantation prevents the allograft dysfunction associated with reflux. Ann Thorac Surg 2011;92:462-8; discussion 468-9.

177. Hoppo T, Jarido V, Pennathur A, et al. Antireflux surgery preserves lung function in patients with gastroesophageal reflux disease and end-stage lung disease before and after lung transplantation. Arch Surg 2011;146:1041-7.

178. Fisichella PM, Davis CS, Lundberg PW, et al. The protective role of laparoscopic antireflux surgery against aspiration of pepsin after lung transplantation. Surgery

2011;150:598-606.

179. Fisichella PM, Davis CS, Lowery E, et al. Pulmonary immune changes early after laparoscopic antireflux surgery in lung transplant patients with gastroesophageal reflux disease. J Surg Res 2012;177:e65-73.

180. Walter S, Gudowius P, Bosshammer J, et al. Epidemiology of chronic Pseudomonas aeruginosa infections in the airways of lung transplant recipients with cystic fibrosis. Thorax 1997;52:318-21.

181. Roby BB, McNamara J, Finkelstein M, et al. Sinus surgery in cystic fibrosis patients: comparison of sinus and lower airway cultures. Int J Pediatr Otorhinolaryngol 2008;72:1365-9.

182. Nunley DR, Grgurich W, Iacono AT, et al. Allograft colonization and infections with pseudomonas in cystic fibrosis lung transplant recipients. Chest 1998;113:1235-43.

183. Holzmann D, Speich R, Kaufmann T, et al. Effects of sinus surgery in patients with cystic fibrosis after lung transplantation: a 10-year experience. Transplantation 2004;77:134-6.

184. Vital D, Hofer M, Boehler A, et al. Posttransplant sinus surgery in lung transplant recipients with cystic fibrosis: a single institutional experience. Eur Arch Otorhinolaryngol 2013;270:135-9.

185. Vital D, Hofer M, Benden C, et al. Impact of sinus surgery on pseudomonal airway colonization, bronchiolitis obliterans syndrome and survival in cystic fibrosis lung transplant recipients. Respiration 2013;86:25-31.

186. Leung MK, Rachakonda L, Weill D, et al. Effects of sinus surgery on lung transplantation outcomes in cystic fibrosis. Am J Rhinol 2008;22:192-6.

Malignancies after lung transplantation

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Abstract: Lung transplantation has become an efficient life-saving treatment for patients with end stage respiratory disease. The increasing good outcome following lung transplantation may be explained by growing experience of transplant teams and availability of potent immunosuppressive drugs. Nevertheless, the latter carries an inherent risk for malignancy besides other common side effects such as systemic hypertension, diabetes and renal dysfunction. Malignancies occur in a smaller proportion of patients but explain for a large proportion of deaths following transplantation. From the first year post-transplantation they will represent the third cause of death with an increasing incidence along post lung transplant survival. In this chapter, we will browse the different types of malignancies arising following lung transplantation. According to the different techniques for lung transplantation, specific types of bronchogenic carcinoma will be described in the explanted lung, in the native lung, and in the graft. Risk factors associated to immunosuppressive therapy, but also to occupational and environmental factors, especially smoking, will be discussed. Eventually, we will strive at integrating recommendations for the treatment of malignancies following lung transplantation.

Keywords: Lung transplantation; malignancies; immunosuppressive regimen; risk factors; bronchogenic carcinoma

Introduction

Relying on a clinical experience of more than 30 years, lung transplantation has become an efficient treatment for patients with end-stage respiratory disease. Overall median survival has reached 5.8 years regardless of the initial respiratory disease and the type of transplantation (1). When considering patients undergoing bilateral lung transplantation, conditional median survival for patients surviving the first year even has reached 9.8 years (1). These increasingly good outcomes may be explained by growing experience of transplant teams in long term management of patients, and availability of potent anti-infectious and immunosuppressive drugs.

While unconturnable to achieve long-term survival, immunosuppressive regimens also carry deleterious consequences for lung transplant recipients. Systemic hypertension arises in 80% of all patients surviving more than 5 years to lung transplantation; renal dysfunction, hyperlipidemia, and diabetes occur in 62%, 52%, and 34% respectively. In comparison to the general population, cardiovascular comorbidities are more frequent in lung transplant recipients but represent the fourth cause of death only (1). On the opposite, malignancies occur in a lower proportion of patients (18% of patients reaching 5 years survival, 28.7% of patients reaching 10 years survival) but are a stronger purveyor of mortality (1). Malignancies (lymphoma and solid organ malignancies)

represent the third cause of death from the first-year post-transplant on (1). The two other main causes of death are graft related: chronic lung allograft dysfunction and infections. From the date of lung transplantation, the proportion of patients dying from malignancy is growing continuously with survival time, ranging from 3% of all patients during the first-year post-transplant to 14.5% after 5 years of survival (1). The opposite trends in comparison to the general population may be explained by specific risk factors related to lung transplantation.

Immunosuppressive regimens

Immunosuppressive therapy has been a key point in the development of lung transplantation: true survival following lung transplantation was only achieved in the early 80s following the discovery of cyclosporine and increasing awareness for the need of a strong immunosuppression to maintain the lung graft and patients' survival (2).

So far, immunosuppressive regimen for lung transplantation is based on a three drugs regimen using calcineurin inhibitors, antimetabolites, and steroids. Two of the three main types of drugs used for lung transplantation carry a potential oncogenic effect of their own. Calcineurins inhibitors (CNI), such as cyclosporine and tacrolimus, inhibit DNA repair and apoptosis in damaged cells, and may support tumor progression by this pathway (3-5). CNI also inhibit cell adhesion, which acts in favor of cell migration or metastatic spread (6,7). Azathioprine determines microsatellite DNA instability in myelodysplastic syndromes and may induce squamous cell carcinoma of the skin (8-10). Nevertheless, there is no evidence that steroids may enhance tumor progression. In specific situations of lymphoma, steroids may even reduce the tumor size and induce cell death.

On the opposite, newer drugs such as mTOR inhibitors have theoretical anti-tumor properties, although no clinical effect has ever been obviated in lung transplant recipients (11-13). While mTOR inhibitors are recommended to treat pleural Kaposi sarcoma in renal transplant recipients, attempts to modify lung transplant patients' regimen with introducing mTOR after solid organ cancer diagnosis did not affect the outcome or modify the natural history of disease (14). Despite some experimental evidence crediting antiproliferative properties to mTOR inhibitors, the relatively low caseload of lung transplant patients with solid organ cancer opposes to any clinical trial evaluating their potential contribution to cancer treatment.

Eventually, immunosuppression is a double-edged sword, which does not only favor development of cancer, but also adversely interferes with the efficacy of systemic antineoplastic treatment. By one aspect, immunosuppression will have deleterious consequences on the clinical state of the patient: renal dysfunction for example jeopardizes the possibilities for chemotherapy. Moreover, there is now experimental evidence showing that systemic treatment such as radiotherapy or chemotherapy are only efficient if they are associated to a competent immune system in the host organism. There is an effective span of time during which the given systemic therapy has its own direct effect on cancer. Once the administration of the therapy is over, the competent immune system maintains the result obtained on the tumor while relapse is observed rapidly in immunosuppressed organisms. The immunosuppressed state of lung transplant recipient may explain for the very short delays of relapse following systemic treatment for cancer (15-17).

Post-transplant lymphoproliferative disease (PTLD)

Regarding the risk for malignancies, immunosuppressive medications used for the regimen of lung transplant recipients will lead patients to a comparable state as HIV infection (18). Malignancies related to viral infections, such as Kaposi sarcomas or viral induced lymphomas, are favored. PTLD is caused by infection with oncogenic viruses, where the Epstein Barr virus (EBV) is the most prominent (19). The risk for PTLD increases with the cumulative level of immunosuppression. The first publication in 1984 showed the relation of PTLD with immunosuppression, but also the potential for regression of the disease when tapering the level of immunosuppression (20). Only a few non-EBV related cases have been reported. The prevalence of EBV infection is high: almost 95% of the general population have been infected, and most often in an asymptomatic fashion during childhood. Most patients will have experienced EBV infection at the time they qualify for lung transplantation. EBV has a tropism for naïve B cells. In the immunocompetent host, EBV infection will summarize as a B cell infection turning B cells to proliferating B blasts, but the reaction of the competent immune system will turn them into B memory cells and let them quiet. On the opposite, immunosuppressive therapy will hamper the control of the immune system over infected B cells and allow them to replicate themselves. EBV DNA load will then be

detectable in the peripheral blood (21). In the rare event of EBV negative patients at the time of lung transplantation, primary EBV infection after the start of immunosuppressive therapy is a serious threat, carrying a higher risk for PTLD (22,23). In both scenarios, there is no validated EBV DNA load threshold to confirm PTLD on a pathognomonic basis (24). Confirmation of definitive diagnosis relies on the same strategy as for any lymphoproliferative disease. Treatment refers to usual chemotherapy protocols as per guidelines but needs to be associated with a tapering of immunosuppressive therapy (25). Comorbidities induced by long-lasting immunosuppression limit the application of chemotherapy.

Skin malignancies

Skin cancer following lung transplantation is another oncologic complication directly related to the strength of immunosuppression (26). Ducloux *et al.* demonstrated a correlation between a low titre of CD4 lymphocytes and the occurrence of skin carcinoma. Different organ transplants require different levels of immunosuppression: accordingly, incidence of skin carcinoma was lower in liver recipients, and higher in hearts transplants when compared to kidney recipients (27,28). Similarly, patients submitted to three-drug regimens had a higher incidence of skin malignancies than recipients submitted to two-drugs regimens (27). According to a review of the UNOS registry, Magruder *et al.* obviated that skin cancer had been diagnosed in 16% out of 18,093 lung transplant recipients, for whom a three-drug regimen is mandatory (29). In comparison, only 7.2% of the same cohort developed any other type of *de novo* malignancy. The majority of skin lesions were squamous cell carcinomas. Co-carcinogenic factors do add to the risk represented by immunosuppressive drugs. These are viral infections with human papillomavirus infections on top, excessive sun exposure, and skin type (30).

There is still a controversy regarding the use of voriconazole; while considered effective to prevent aspergillosis, it acts as a potent photosensitizer (31,32). Several studies obviated a higher incidence of skin carcinoma, especially of non-melanoma type, to the duration of voriconazole therapy. However, McLaughlin *et al.* identified confounding demographic and clinical factors which outweigh the relation between use of voriconazole and skin cancer to a non-significant level in lung and heart-lung recipients (33). Male gender, sun exposure, advanced age, history of COPD, and history of

immune disorder were independent risk factors for non-melanoma skin cancer after lung transplantation (33).

Age at the time of lung transplantation

In the general population, risk for cancer increases with age. Passing the threshold of 60 years of age, the risk for lung cancer doubles from 0.95% to 2.35% in men, and from 0.79% to 1.75% in women (34). The probability of developing cancer of any kind reaches 6% when patients cross the age of 50 years and reaches 14% when crossing the age of 60 (35). Gender related malignancies even have lower age limits: the probability for breast cancer in women or prostate cancer in men dramatically increases when crossing the threshold of 40 years of age (36). In the meantime, the proportion of lung transplant recipients older than 40 years has increased with time to more than 40%. In addition, improved survival by era now leads younger recipients to cross the age landmarks. At least 30% of lung transplant recipients will live longer than 60 years of age (1).

Occupational risk factors and lung disease

Lung disease requiring lung transplantation and lung cancer share some common occupational and environmental risk factors.

Smoking history

Smoking is a major occupational risk factor. COPD and emphysema are the most common indications for lung transplantation in the ISHLT registry (1). In the recording of recipient's characteristics, COPD/emphysema is set apart from alpha-1 antitrypsin deficiency and represents 31% of all lung transplants (1). COPD/emphysema occurrence in patients is highly related to smoking history. Even in COPD/asthma overlap syndrome (ACOS), smoking history remains an important risk factor (37-39).

Recipient's and/or donor's smoking history is one of the main risk factors for primary lung carcinoma in lung transplant recipient. Primary lung carcinoma has been described in the native lung after single-lung transplantation, as well as in the allograft following single- or bilateral lung transplantation (40). In both cases, smoking history was identified as a risk factor. But smoking history is also a known risk factor for other malignancies such as bladder and urinary tract cancer, esophagus, pancreas, oral cavity, larynx, kidney, stomach, uterine cervix and acute

myeloid leukemia (41). Smoking history is responsible for 30% of all cancer deaths in the United States (41). Approximately 60% to 70% of all candidates for lung transplantation have experienced smoking history (42,43). Even in CF patients, some studies report up to 16% of former smokers (43).

Occupational and environmental factors

Some other occupational or environmental risk factors are related with the onset of lung fibrosis and possible lung carcinoma. Asbestosis and silicosis are both interstitial lung diseases that may lead to end-stage lung disease requiring transplantation and are risk factors for lung cancer at the same time. Even when not related to an environmental risk factor, lung fibrosis and lung disease related to systemic sclerosis may evolve to lung carcinoma based on a common ground of molecular pathogenic pathways (44). Fibrosis and lung interstitial disease represent the second most frequent indication for lung transplantation. All-together, COPD and fibrosis together represent more than 70% of all lung transplants each year and are a category of increased risk for lung cancer (1).

Donor risk factors

Risk for cancer brought in by the donor is due either to pre-existing risk factors (especially risk factors for lung cancer), or to an evolutive neoplastic disease at the time of organ donation.

In the current context of shortage of donor lungs, it is well admitted to accept organs from donors with a documented exposure to risk factors such as moderate smoking or mineral particle inhalation, provided that donor work-up has excluded any abnormality. Lung cancer arising in the transplanted lung is so far an exceptional complication, concerning less than 2% of recipients (40). The appraisal of the real risk for cancer related to donor's smoking is so far controversial, but might become more obvious owing to steadily increasing survival after transplant. However, most published events of lung cancer after transplantation have been observed on lungs harvested from former smokers (45,46).

Harvesting lungs from a donor with an active malignant tumour is an exceptional event. Two situations of brain death might be distinguished: either brain death occurs from an inner neural cause including brain tumors, or the brain death occurs from an outer neural cause. In the latter,

donors with a known malignancy will be deemed unsuitable for solid organ donation. Indeed, most often, when a tumor is discovered during work-up or during the harvest procedure, the latter is stopped and all transplantations are cancelled. Only a few isolated cases of ongoing transplantation have been reported. The reasons to proceed with transplantation have been (1) high emergency status of the recipient and low estimated risk for metastases to the lung, (2) cancer which has not been identified at the time of lung harvesting, and (3) failure of recognizing cancer at frozen section analysis during harvest procedure (47). On theoretical grounds, circulating tumor cells at the time of harvest, or existing micrometastases in the donor organ might evolve towards gross metastases in the recipient subjected to immunosuppressive therapy (47). Considering solid organ donors with brain tumors, there is a proved case of a single donor with an active glioblastoma having transmitted tumors to three separate recipients (liver, kidney and lung) published in international literature (48). To prevent this dreadful complication, thorough clinical work-up before, and careful surgical examination of the whole donor during solid organ retrieval are mandatory (49). Some authors have proposed specific additional screening protocols for potential donors to rule out metastases in the harvested organs. Part of their recommendations may be easily applied such as millimetric body CT scan in all potential donors. But another part like performing systematic colonoscopy in donors older than 60 years may increase risks for donor loss (perforation) and costs (49). Though highly relevant for potential solid organ recipients, these recommendations still need to be validated on prospective clinical series of donor selection (49).

In the case of brain dead donors in which brain death is related to a brain tumor, the world health organization (WHO) classification based on the aggressiveness of central nervous system (CNS) tumors may help decide whether the donor should be proposed for solid organ harvesting or not (50). The risk of transmission is separated in 4 classes provided the pathological nature of the CNS tumor is known. According to the UNOS registry, low grade tumors have a low risk of transmission (0.1% to 1%) whereas high grade tumor (III or IV in the WHO classification) have a high risk of transmission (over 10%) (50-52). Despite low risk for grade I and II tumors (0.1% to 1%), the recipient should always be informed before performing solid organ transplantation (53). Despite the opportunity of accepting donors with low grade CNS tumors, only a 50% to 60% of patients dying from CNS tumors are accepted as solid

organ donor representing 1% of all donors (54). This fact may come from donors with CNS tumors for which a recent pathologic diagnose is not available: either brain death is inaugural from a CNS tumor and pathologic diagnose was never made, or the donor has a medical story of CNS tumor with ancient pathologic examination but no details are available on the recent history of the tumor and the possibility of evolving towards a higher-grade tumor (53). In the setting of organ shortage, Beigee *et al.* propose to search for pathology results on the CNS tumors at the time of solid organ harvesting starting with a brain autopsy (53). Brain autopsy in the brain-dead heart beating donor is performed at first to send part or whole of the tumor to pathologic examination while the solid organ harvesting is carried out. In their publication, Beigee *et al.* report a 45 to 50 minutes process for pathologic diagnose on the CNS tumor. The short effective span of time enables rapid decision making on whether the transplantation process should be carried out or whether the harvested organs should be discarded. In their publication, brain autopsy first enabled the authors to enlarge their donor pool with 21 solid organs that would otherwise have been discarded facing the uncertain diagnose of the reported CNS tumors (53). One step further, in their retrospective review, Kashyap *et al.* evidenced no survival difference between their liver transplant recipients would they be transplanted with an organ from a CNS brain dead donor or not, whatever the grade of the involved CNS tumor (48). Among 42 liver transplant recipients from CNS donors, only one experienced recurrence from the CNS tumor but the patient eventually died from another cause (48). Liver transplantation requires a lower immunosuppressive regimen in comparison to lung transplantation, but in the end, clinical evidence may plead for a more tolerant attitude when considering CNS tumor brain dead donors. Indeed, when considering the ability of CNS tumors for metastatic spread, lung metastases are a rare occurrence. Less than 2% of all patients with CNS tumors present with extra-neural metastases in the whole medical history of the CNS tumor. Moreover, lung metastases occur in the setting of an end stage evolutive CNS tumor with obvious clinical and radiological signs (54,55).

Scenarios for lung cancer in lung transplant recipients

There are at least 3 different clinical situations: (I) lung cancer discovered on the explanted lung, (II) lung cancer in the native lung after single-lung transplantation, and (III) lung cancer in the transplanted lung of either type (40).

Lung cancer discovered on the explanted lung may be completely unexpected, but some recipients are accepted on waiting list with an undetermined, stable and PET negative nodule. Discovery of lung cancer on the explanted lung naturally arises anxiety about the potential for recurrence after transplantation. On the opposite, one might also consider that explantation of the lung is a potentially curative treatment of early stage cancer. The prognosis is determined by lymph node stage. However, node staging implicates complete node dissection, which is obviously not performed during lung transplantation. As a consequence, prognosis is uncertain in this category of patients. Nevertheless, there is some evidence that prognosis of early stage lung cancer does not differ from non-transplanted patients. On the opposite, prognosis is poor in patients with lymph node involvement, despite adjuvant chemotherapy (40).

The second scenario is bronchogenic carcinoma occurring in the native lung following single-lung transplantation. Single-lung transplantation is indicated for patients without chronic suppurative lung disease, and preferentially without pulmonary hypertension. In other words, most of the patients undergoing single-lung transplantation suffer from either COPD patients or fibrosis. Single-lung transplantation leaves behind a sick lung that has been exposed to risk factors for cancer, and smoking in particular; in addition, risk for lung cancer increases in case of idiopathic fibrosis, regardless of smoking history. Post-transplant immunosuppressive therapy adds to the risk. Bronchogenic carcinoma of the native lung is observed in 5–10 % of single-lung transplant recipients: as such, the risk for bronchogenic carcinoma is a 6-fold higher in comparison to patients undergoing bilateral lung transplantation (40,56)

Nevertheless, even bilateral lung transplantation does not wave away the risk for lung cancer in the graft. Bronchogenic carcinoma is observed in 1–2% of double lung transplant recipients and may arise from the graft as from the recipient himself. Spencer *et al.* demonstrated with repeated bronchial epithelium biopsies following bronchial suture that cells issued from the recipient bronchial epithelium are migrating towards the donor bronchi to form a mosaic with donor epithelial cells in up to 20% of the epithelial surface of the graft (57). These recipient cells may carry risk owing to previous smoking history of the recipient. The other origin for bronchogenic carcinoma

comes from donor cells; there is so far no evidence defining a set-off in the quantification of smoking history. In some rare cases, the donor or recipient origin of cancer cells has been documented with *in situ* fluorescence hybridization assays, showing an almost even distribution.

Treatment of lung cancer after transplantation follows the guidelines applied to any patient with lung cancer. For early stage disease, surgery with curative intent should be considered. However, immunosuppressive therapy introduces a double limitation. As previously discussed, immunosuppressive drugs limit the efficiency of adjuvant therapies (15-17). Further, immunosuppressive drugs may lead to specific comorbidities, which may oppose to surgery and/or chemotherapy. Eventually, as the occurrence of cancer following lung transplantation also increases with duration of survival, the patient might suffer from chronic lung allograft dysfunction at the time of cancer diagnosis and be unfit for surgery on functional grounds.

Final recommendations

There aren't any specific recommendations available for malignancies following lung transplantation.

Considering the direct toxicity of immunosuppressive regimen, but also to the potent oncologic consequences, the equilibrium between benefits and adverse effects is critical. According to the current state of art, lung transplant recipients require a relatively harsh immunosuppression relying on a three-drug regimen (antimetabolite, steroids, and CNI). But in some practices, the regimen may be reduced to two drugs by suppressing the antimetabolite. Besides, use of inhibitors of mTOR during the treatment of malignancies is still matter of debate. The question whether mTOR inhibitors should be added to the immunosuppressive regimen, or if they might substitute to one of the drugs, still needs to be answered with a dedicated trial.

Similarly, there are no recommendations whether the patient should undergo screening programs before lung transplantation or at the time when immunosuppression is started (58). Several authors describing lung cancer in the explanted lung made a retrospective review of chest CT scans of the patients just before lung transplantation. Only a few cases could have been diagnosed or at least suspected before lung transplantation. In most of the cases, there were unspecific findings suitable with infectious disease or lung consolidation, especially in patients with fibrosis (59,60).

Once cancer has been diagnosed on the explanted lung,

some author completed the staging with a complementary lymph node dissection performed through mediastinoscopy or even thoracoscopy. Adjuvant treatment was given following the usual guidelines for lung cancer.

If cancer has declared after lung transplantation, curative resection is the optimal choice, provided that respiratory function allows proceeding, and that comorbidity index is low. Obviously, prognosis is best for early stage.

Prevention of skin cancer relies on patients' education avoiding direct sun exposure, protecting the skin with sunscreens and appropriate clothing. A dermatologist should perform skin surveillance regularly for early detection and excision of premalignant lesions (30). Treatment should follow the usual guidelines for skin carcinoma. In case of melanoma, immunotherapy and interferon therapy may be applied according to the compatibility with immunosuppressive drugs, within the framework of a multidisciplinary team.

PTLD treatment is achieved by tapering the level of immunosuppression, associated to classic chemotherapy schemes. Some authors add mTOR inhibitors to reduce other associated drugs. In the few patients without EBV infection before transplantation, anti-viral therapy should be discussed to prevent primo-infection as it carries an even higher risk for PTLD.

For any other type of cancer, comprehensively speaking, medical work up and treatment should be performed according to the valid actual guidelines with respect to the suspected type of cancer.

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Footnote

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References

1. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant 2015;34:1264-77.
2. Reitz BA, Wallwork JL, Hunt SA, et al. Heart-

lung transplantation: successful therapy for patients with pulmonary vascular disease. N Engl J Med 1982;306:557-64.

3. Ahlers C, Kreideweiss S, Nordheim A, et al. Cyclosporin A inhibits Ca2þmediated upregulation of the DNA repair enzyme DNA polymerase beta in human peripheral blood mononuclear cells. Eur J Biochem 1999;264:952-9.

4. Pritchard DE, Singh J, Carlisle DL, et al. Cyclosporin A inhibits chromium(VI)-induced apoptosis and mitochondrial cytochrome c release and restores clonogenic survival in CHO cells. Carcinogenesis 2000;21:2027-33.

5. Hortelano S, López-Collazo E, Boscá L. Protective effect of cyclosporin A and FK506 from nitric oxide-dependent apoptosis in activated macrophages. Br J Pharmacol 1999;126:1139-46.

6. Hojo M, Morimoto T, Maluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999;397:530-4.

7. Mohri T, Kameshita I, Suzuki S, et al. Rapid adhesion and spread of non-adherent colon cancer Colo201 cells induced by the protein kinase inhibitors, K252a and KT5720 and suppression of the adhesion by the immunosuppressants FK506 and cyclosporin A. Cell Struct Funct 1998;23:255-64.

8. Kasiske BL, Snyder JJ, Gilbertson DT, et al. Cancer after kidney transplantation in the United States. Am J Transplant 2004;4:905-13.

9. Swann PF, Waters TR, Moulton DC, et al. Role of postreplicative DNA mismatch repair in the cytotoxic action of thioguanine. Science 1996;273:1109-11.

10. Offman J, Opelz G, Doehler B, et al. Defective DNA mismatch repair in acute myeloid leukemia/ myelodysplastic syndrome after organ transplantation. Blood 2004;104:822-8.

11. Hussein MM, Mooij JM, Roujouleh HM. Regression of post-transplant Kaposi sarcoma after discontinuing cyclosporin and giving mycophenolate mofetil instead. Nephrol Dial Transplant 2000;15:1103-4.

12. Fernández A, Marcén R, Pascual J, et al. Conversion from calcineurin inhibitors to everolimus in kidney transplant recipients with malignant neoplasia. Transplant Proc 2006;38:2453-5.

13. Chiurchiu C, Carreño CA, Schiavelli R, et al. Results of the conversion to everolimus in renal transplant recipients with posttransplantation malignancies. Transplant Proc 2010;42:277-9.

14. Stallone G, Schena A, Infante B, et al. Sirolimus for

15. Apetoh L, Tesniere A, Ghiringhelli F, et al. Molecular interactions between dying tumor cells and the innate immune system determine the efficacy of conventional anticancer therapies. Cancer Res 2008;68:4026-30.

16. Taniguchi K, Nishiura H, Yamamoto T. Requirement of the acquired immune system in successful cancer chemotherapy with cis-diamminedichloroplatinum (II) in a syngeneic mouse tumor transplantation model. J Immunother 2011;34:480-9.

17. Zitvogel L, Apetoh L, Ghiringhelli F, et al. The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008;118:1991-2001.

18. Serraino D, Piselli P, Busnach G, et al. Risk of cancer following immunosuppression in organ transplant recipients and in HIV-positive individuals in southern Europe. Eur J Cancer 2007;43:2117-23.

19. Verschuuren EAM. Post-Transplant lymphoproliferative syndrome and other malignancies. Eur Respir Mon 2009;45:226-37.

20. Starzl TE, Nalesnik MA, Porter KA, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. Lancet 1984;1:583-7.

21. Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. N Engl J Med 2004;350:1328-37.

22. Walker RC, Paya CV, Marshall WF, et al. Pretransplantation seronegative Epstein-Barr virus status is the primary risk factor for posttransplantation lymphoproliferative disorder in adult heart, lung, and other solid organ transplantations. J Heart Lung Transplant 1995;14:214-21.

23. Haque T, Thomas JA, Falk KI, et al. Transmission of donor Epstein-Barr virus (EBV) in transplanted organs causes lymphoproliferative disease in EBV-seronegative recipients. J Gen Virol 1996;77:1169-72.

24. Stevens SJ, Verschuuren EA, Pronk I, et al. Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. Blood 2001;97:1165-71.

25. Bakker NA, Verschuuren EA, Erasmus ME, et al. Epstein-Barr virus-DNA load monitoring late after lung transplantation: a surrogate marker of the degree of immunosuppression and a safe guide to reduce immunosuppression. Transplantation 2007;83:433-8.

26. Bouwes Bavinck JN, Hardie DR, Green A, et al.

The risk of skin cancer in renal transplant recipients in Queensland, Australia. A follow-up study. Transplantation 1996;61:715-21.

27. Jensen P, Hansen S, Møller B, et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol 1999;40:177-86.

28. Frezza EE, Fung JJ, van Thiel DH. Non-lymphoid cancer after liver transplantation. Hepatogastroenterology 1997;44:1172-81.

29. Magruder JT, Crawford TC, Grimm JC, et al. Risk Factors for De Novo Malignancy Following Lung Transplantation. Am J Transplant 2017;17:227-38.

30. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. N Engl J Med 2003;348:1681-91.

31. Feist A, Lee R, Osborne S, et al. Increased incidence of cutaneous squamous cell carcinoma in lung transplant recipients taking long-term voriconazole. J Heart Lung Transplant 2012;31:1177-81.

32. Zwald FO, Spratt M, Lemos BD, et al. Duration of voriconazole exposure: an independent risk factor for skin cancer after lung transplantation. Dermatol Surg 2012;38:1369-74.

33. McLaughlin JM, Equils O, Somerville KT, et al. Risk-adjusted relationship between voriconazole utilization and non-melanoma skin cancer among lung and heart/lung transplant patients. Transpl Infect Dis 2013;15:329-43.

34. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300. Erratum in: CA Cancer J Clin 2011;61:133-4.

35. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017;67:7-30.

36. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.

37. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. N Engl J Med 2015;373:1241-9.

38. Gelb AF, Nadel JA. Understanding the pathophysiology of the asthma-chronic obstructive pulmonary disease overlap syndrome. J Allergy Clin Immunol 2015;136:553-5.

39. Hines KL, Peebles RS Jr. Management of the Asthma-COPD Overlap Syndrome (ACOS): a Review of the Evidence. Curr Allergy Asthma Rep 2017;17:15.

40. Olland AB, Falcoz PE, Santelmo N, et al. Primary lung cancer in lung transplant recipients. Ann Thorac Surg 2014;98:362-71.

41. Curran JN, Garrett-Mayer E, Carpenter MJ, et al. Cigarette smoking and the risk of dying from tobacco-related malignancies by race. Anticancer Res 2011;31:3891-5.

42. Hellemons ME, Agarwal PK, van der Bij W, et al. Former smoking is a risk factor for chronic kidney disease after lung transplantation. Am J Transplant 2011;11:2490-8.

43. Evon DM, Burker EJ, Sedway JA, et al. Tobacco and alcohol use in lung transplant candidates and recipients. Clin Transplant 2005;19:207-14.

44. Daniels CE, Jett JR. Does interstitial lung disease predispose to lung cancer? Curr Opin Pulm Med 2005;11:431-7.

45. Beyer EA, DeCamp MM, Smedira NG, et al. Primary adenocarcinoma in a donor lung: evaluation and surgical management. J Heart Lung Transplant 2003;22:1174-7.

46. Minai OA, Shah S, Mazzone P, et al. Bronchogenic carcinoma after lung transplantation: characteristics and outcomes. J Thorac Oncol 2008;3:1404-9.

47. Desai R, Collett D, Watson CJ, et al. Estimated risk of cancer transmission from organ donor to graft recipient in a national transplantation registry. Br J Surg 2014;101:768-74.

48. Kashyap R, Ryan C, Sharma R, et al. Liver grafts from donors with central nervous system tumors: a single-center perspective. Liver Transpl 2009;15:1204-8.

49. Hassanain M. Novel guidelines for organ donor cancer screening. Ann Transplant 2014;19:241-7.

50. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97-109. Erratum in: Acta Neuropathol 2007;114:547.

51. Cancer Facts & Figures 2018, American cancer society website. Available online: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2018/cancer-facts-and-figures-2018.pdf. Accessed April 2018.

52. Zülch KJ. Principles of the new World Health Organization (WHO) classification of brain tumors. Neuroradiology 1980;19:59-66.

53. Sadegh Beigee F, Shahryari S, Mojtabaee M, et al. Pathology Results at Autopsy in Brain-Dead Patients with Brain Tumors. Exp Clin Transplant 2017;15:113-5.

54. Lun M, Lok E, Gautam S, et al. The natural history of extracranial metastasis from glioblastoma multiforme. J Neurooncol 2011;105:261-73.

55. Beauchesne P. Extra-neural metastases of malignant gliomas: myth or reality? Cancers (Basel) 2011;3:461-77.

56. Dickson RP, Davis RD, Rea JB, et al. High frequency of bronchogenic carcinoma after single-lung transplantation. J Heart Lung Transplant 2006;25:1297-301.

57. Spencer H, Rampling D, Aurora P, et al. Transbronchial biopsies provide longitudinal evidence for epithelial chimerism in children following sex mismatched lung transplantation. Thorax 2005;60:60-2.

58. Strollo DC, Dacic S, Ocak I, et al. Malignancies incidentally detected at lung transplantation: radiologic and pathologic features. AJR Am J Roentgenol 2013;201:108-16.

59. de Perrot M, Fischer S, Waddell TK, et al. Management of lung transplant recipients with bronchogenic carcinoma in the native lung. J Heart Lung Transplant 2003;22:87-9.

60. de Perrot M, Chernenko S, Waddell TK, et al. Role of lung transplantation in the treatment of bronchogenic carcinomas for patients with end-stage pulmonary disease. J Clin Oncol 2004;22:4351-6.

Infections after lung transplantation

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Abstract: The good clinical result of lung transplantation is constantly undermined by the high incidence of infection, which negatively impacts on function and survival. Moreover, infections may also have immunological interactions that play a role in the acute rejection and in the development of chronic lung allograft dysfunction. There is a temporal sequence in the types of infection that affects lung allograft: in the first postoperative month bacteria are the most frequent cause of infection; following this phase, cytomegalovirus and Pneumocystis carinii are common. Fungal infections are particularly feared due to their association with bronchial complication and high mortality. Scrupulous postoperative surveillance is mandatory for the successful management of lung transplantation patients with respect to early detection and treatment of infections. This paper is aimed to address clinicians in the management of the major infectious complications that affect the lung transplant population.

Keywords: Bacterial infections; lung transplantation; mycoses; postoperative complications; virus diseases

Introduction

Infection is a common complication after lung transplantation, its recognition may be difficult, and signs and symptoms may sometimes be misleading. Since lifelong immunosuppression is mandatory to prevent acute and chronic rejection, immune system impairment contributes to increased patient vulnerability to infectious agents. At least four clinical scenarios are classically indicated as possible high-risk situations: recipients can host infections from a wide range of microorganisms (especially among patients with cystic fibrosis) or they may become colonized with nosocomial organisms; lung grafts could promote the transmission of infections from donors and, finally, transplanted patients are prone to major infection from agents that are relatively innocuous in an immunocompetent host (1).

Time is a determining factor for the development of infection after lung transplantation; infections are the second cause of mortality within the first 30 days after transplantation (19.2%) but reach the first position (37.3%) between 30 days and 1 year. After one year from surgery, the mortality rate for infections decreases modestly, though always remaining among the main causes of graft failure (2). Time also affects the category of infections the transplant patient can develop: in the first post-operative month, the etiologic cause of the infection is often to be found in germs present in the donor or recipient. Nosocomial infections are frequent in this period, as are infections related to technical problems [catheter infections, surgical site infections (SSI), dehiscence of bronchial anastomoses]. From 1 to 6 months after transplantation, opportunistic agents as well as reactivation of latent infections are common. Six months after transplantation, infections due to community-acquired pathogens are the major concern (1).

The diagnosis of infection is a difficult task in lung transplantation since symptoms such as fever, fatigue, loss of appetite, night sweats, chills, and pain may be unremarkable or absent due to immunosuppressive therapy. Loss of lung function may be observed in lung infection, but is also common in acute and chronic rejection. In addition, white blood cell count is frequently altered from concomitant corticosteroid use. On the contrary, evidence supports the utility of serum procalcitonin to diagnose infections among solid organ transplant recipients, with accuracy rates similar to those of the general population (3).

Considering that pneumonia is the most common form of infection after lung transplantation, chest computed tomography (CT) is a useful instrument for the diagnosis of most of these disorders. Parenchymal consolidations, pleural effusions, micronodules, and interlobular septa thickening are CT common findings in bacterial infections. Invasive aspergillosis presents a CT pattern consisting of pulmonary opacities, with or without excavation, surrounded by a ground glass halo. Ground glass opacities with scattered micronodules (tree-in-bud pattern), bronchiectasis and consolidations are the most common CT findings in viral infections. Pneumocystis infection shows diffuse ground glass opacities that often spare the sub-pleural portion of the lung; focal consolidations, interlobular septa thickening ("crazy paving" pattern) and micronodules are common. Cystic lesions in the upper lobes complicated by pneumothorax or pneumomediastinum are also possible in opportunistic Pneumocystis infection (4).

Bronchoalveolar lavage and transbronchial biopsies have an excellent diagnostic yield for bacteria and opportunistic pathogens. Given that Aspergillus colonization is a demonstrated risk factor for severe airway complications and invasive aspergillosis, surveillance with flexible bronchoscopy is advisable during the first year after transplantation (5).

Pretransplantation infections and vaccinations

Before transplantation, it is essential to identify patient airway colonization and to investigate former infectious disease history. Such information is usually available in cystic fibrosis patients, given constant monitoring. Patients with other indications for transplantation should be carefully investigated for possible infectious diseases, even in latent form. At the time of listing, a wide panel of laboratory tests is recommended for candidates; such panel includes serological tests for cytomegalovirus (CMV),

Epstein-Barr virus (EBV), hepatitis B (HBV) and C viruses, herpes simplex virus (HSV), human immunodeficiency virus, *Treponema pallidum* and varicella-zoster virus (VZV). Furthermore, bronchoalveolar lavage may provide information on bronchial flora to guide antibiotic therapy before and/or after transplantation. Patients who are discovered to be methicillin-resistant *Staphylococcus aureus* carriers should receive an eradication protocol for the upper and/or lower respiratory tract (6).

If the estimated rate of methicillin-resistant *Staphylococcus aureus* colonization in patients evaluated for solid organ transplantation is 8.5%, the estimated vancomycin-resistant enterococcus colonization rate reaches 12% of candidates (7). The driver for this high prevalence is exposure to healthcare settings; therefore, meticulous respect for hygiene rules among health professionals must be constantly stressed. Vancomycin-resistant enterococcus is not considered highly pathogenic but careful surveillance is mandatory especially after transplantation. Finally, carbapenem-resistant enterobacteriaceae (including carbapenemase producing *Klebsiella pneumoniae*—KPC) are highly resistant Gram-negative bacteria that can colonize the recipient bowel exposing the patient to severe infection with high mortality rates. Currently, no decolonization strategies have been developed but fecal microbiota transplantation is a procedure that creates hope for the future (8).

History of possible tuberculosis (TB) must be carefully investigated; recent migration flows must increase the degree of alert, even towards low risk citizens from western countries. Should active TB be identified in a potential lung transplant candidate, proper therapy should be completed prior to listing. Tuberculin skin testing and/or QuantiFERON Gold TB test is recommended in all patients at listing.

Mycobacterium abscessus complex, a group of rapidly growing non-tuberculous mycobacteria (NTM), has emerged as a major problem, particularly in cystic fibrosis candidates for lung transplantation. According to the American Thoracic Society and the Infectious Diseases Society of America criteria, the diagnosis of non-tuberculous mycobacterium disease must include radiological signs (excavated opacities and/or solid nodules, multifocal bronchiectasis, "tree-in-bud") and positive culture from bronchoalveolar lavage or lung biopsy or at least two separated expectorated sputum samples (9). Many centers consider patients with *Mycobacterium abscessus* disease not eligible for lung transplantation. Centers that accept patients with this condition require the infection be under

control and that treatment be tolerated by the patients. In addition, the surgical procedure needs special precautions: complete hilar and mediastinal lymphadenectomy, pleural cavity washing with amikacin solution and change of surgical gloves after pneumonectomy. Postoperative antimycobacterial antibiotic treatment should last for at least 1 month, if not lifelong (10).

Severe disease and graft rejection could affect young transplant recipients due to infection from vaccine-preventable agents; despite this evidence, immunization is sometimes inadequate in young patients who are listed for lung transplantation mainly for fear of vaccine-related side effects. It is advisable that transplant centers develop a specific vaccination guideline or, at least, follow the national vaccination program prior to lung transplantation. HBV, pneumococcal and meningococcal vaccinations are among the uncommon immunizations that should be implemented, bearing in mind that a time lapse of at least 3 months is advisable between vaccination and transplantation. Monitoring of immunization to vaccine-preventable infections before transplantation is quite common among transplantation centers and offers the opportunity to proceed with opportune vaccinations if needed (11). Finally, influenza vaccination is highly recommended both before and after transplantation for the patient as well as for family contacts.

SSI and antibacterial prophylaxis

Following CDC 1992 indications, infections after surgery are defined as SSI and classified into superficial incisional SSI, deep incisional SSI and organ and/or space SSI (12). To prevent SSI following lung transplantation meticulous attention to asepsis rules is mandatory: accurate skin preparation with products containing iodophors or chlorhexidine gluconate in aqueous or alcohol-based solution is needed; the sterile field should be prepared as close as possible to the time of use; once sterile drapes are placed they should not be rearranged. Renewal of sterile drapes may be considered after a number of hours of surgery. In recipients with a high bacterial burden, appropriate washing of the pleural cavity as well as the recipient trachea and bronchial stumps with iodophors aqueous solution or antibiotic solution prior to implantation of the graft is indicated. Anesthesiologists are requested to substitute suction catheters and the bronchoscope after the graft implantation, particularly in recipients with prior airway colonization. Closed wound suction units with a suitable catheter are advisable in patients with thick adipose

tissue or large breasts, to prevent incisional SSI.

There is a sufficient consensus for the use of perioperative antibiotics in general thoracic surgery even though the appropriate duration of the antibiotic administration is not fully shared (13). Unfortunately, neither guidelines nor standard treatments exist regarding the choice of perioperative antibiotic in lung transplantation, but regular prophylaxis is recommended for all recipients. For patients without septic disease who receive a graft free from documented bacterial colonization, ceftazidime is considered the first choice; administration should begin before the incision and be repeated 3 times a day according to renal function. Generally, a period of 48–72 hours is considered appropriate. Piperacillin-tazobactam or cefepime are good alternatives to ceftazidime; levofloxacin could be used in patients with beta-lactam allergy. Vancomycin is generally used, in association with ceftazidime, to cover Gram-positive bacteria. Like ceftazidime, vancomycin should be started before incision; administration should continue twice a day for 48–72 hours. When the postoperative course is uneventful and intubation time is short, the initial antibiotic pattern can be interrupted as scheduled, but it is advisable to perform regular bronchoscopic controls (every seven days for the first month in our department). If the postoperative course presents complications, antibiotic prophylaxis should be continued and eventually adapted to identified pathogens. Patients who receive lungs from donors infected with known pathogens should obviously be treated with appropriate antibiotic scheme for a reasonable period (at least until two consecutive bronchoalveolar lavages are negative).

Patients who receive lung transplantation for high bacterial burden conditions (cystic fibrosis, bronchiectasis, etc.) require special attention. Generally, continuation of patient specific antibiotic patterns is advisable for at least 30 days; in case of highly resistant germs, treatment may be continued following discharge at home for another 30–60 days. Special attention should be paid to *Burkholderia cenocepacia* carriers due to the high probability of postoperative uncontrollable systemic virulence. Currently, the majority of transplant centers believe that patients harboring this specific species of Burkholderia are not fit to receive lung transplantation (14).

Antifungal prophylaxis

Lung transplant recipients have a higher risk of fungal infections than other solid-organ recipients. Aspergillus

is the most frequent pathogen in lung transplantation whereas Candida is the leading pathogen in other solid-organ transplants. Seldom, other fungi can also cause severe infections; among them Cryptococcus, Fusarium, Scedosporium, mucormycetes and endemic agents (Blastomyces, Coccidioides and Histoplasma).

Invasive Aspergillosis is one of the most feared infectious complications after pulmonary transplantation; it occurs generally within 1 year, but it can affect patients up to 3 years after transplantation. Bronchial anastomotic infections commonly occur within the first 3 months after transplantation and may evolve towards Aspergillus ulcerative tracheobronchitis, which is among the worst complications of the surgical procedure.

Antifungal prophylaxis efficacy is well documented in liver transplantation, on the contrary, there is still a lack of evidence on the optimal strategy in lung transplantation. As a consequence, prophylaxis strategies for reducing fungal infection are heterogeneous among lung transplantation centers. A systematic review and meta-analysis, collecting seven studies (mostly retrospective and monocentric) published in 2016, concluded that universal fungal prophylaxis compared with no or targeted prophylaxis reduces the incidence of invasive fungal infection in lung transplant patients (15).

Administration of a systemic azole, inhaled antifungal medication or the combination of the two strategies are possible approaches to universal antifungal prophylaxis. On the other hand, the administration of antifungal agents to high risk patients (cystic fibrosis, complicated postoperative period, advanced donor age, induction therapy, airway ischemia) or to patients with documented airway colonization are defined as target therapy and preemptive therapy, respectively.

Systemic administration of voriconazole or posaconazole is the most rational strategy for universal prophylaxis, but hepatotoxicity and drug interactions should be carefully monitored. The duration of such prophylaxis is also heterogeneous among centers and it ranges between 6 to 12 months. Liposomal Amphotericin B is the drug generally used for inhaled-strategy prophylaxis; daily administration for 1–2 weeks followed by once weekly for 1–3 months is one of the several protocols in use. In our center, we prefer universal prophylaxis with voriconazole for 6 months with the addition of preemptive therapy when needed.

Pneumocystis pneumonia (PCP) prophylaxis

Pneumocystis are unicellular fungi that constitute important pathogens that may cause severe disease in immunocompromised hosts, defined as PCP. It should be noted that the risk for PCP is higher among lung transplant recipients compared with other organ recipients. Fortunately, since the 1980's, therapy with trimethoprim-sulfamethoxazole, or parenteral pentamidine, has dramatically improved the outcomes of patients who develop PCP.

Lifelong universal prophylaxis with trimethoprim-sulfamethoxazole once-daily or thrice-weekly is highly recommended. For patients who are intolerant to trimethoprim-sulfamethoxazole inhaled pentamidine is an alternative treatment; if the intolerance was discovered before listing, desensitization should be attempted.

CMV

CMV is a beta herpes virus and, after bacterial pneumonia, is the second most common infection in lung transplant recipients (16), being one of the main cause of morbidity and mortality in these patients. It has been associated with tissue injury and infection, and some authors indicate CMV as a risk factor for acute and chronic rejection (17,18); finally, CMV may play an immunomodulatory role promoting several other opportunistic infections (19).

Clinical manifestations

Individuals are primarily infected through physical contact, which involves direct inoculation of infected cells or body fluids, and then harbour CMV for life. CMV generally produces an asymptomatic or a mild acute illness in immunocompetent patients, whilst it can be the cause of severe non-specific syndrome (with fever, malaise, myalgias, arthralgias, leukopenia and thrombocytopenia) and/or organ disease (pneumonia, encephalitis, retinitis, hepatitis or colitis with ulcerations) in an immunosuppressed host.

After lung transplantation, CMV infection may occur in different ways (16):

❖ By transmission from the graft from a CMV seropositive donor;
❖ By reactivation of latent infection in a previously seropositive recipient;
❖ By contact with a CMV infected individual;
❖ By transfusion of hemocomponents from a CMV seropositive blood donor.

The lung has been identified as a major site of CMV latency and recurrence; lung transplantation is associated

with the transfer of a larger CMV load than other solid organs, increasing the risk of CMV infection and disease in these recipients.

Following the American Transplantation Society definitions, CMV infection is defined by the evidence of CMV replication regardless of symptoms, whilst CMV disease is characterised by the presence of a constitutional symptomatic syndrome or tissue-invasive disease (20).

Diagnosis

At present several diagnostic tests are available for CMV: serology, qualitative and quantitative polymerase chain reaction (PCR), pp65 antigenemia, culture, and histopathology.

CMV serology should be performed before transplantation on both the organ donor (D) and the recipient (R), since their serostatus is a key predictor of infection risk and management, with seronegative recipients of seropositive organs (D+/R–) having the highest risk, D+/R+ and D–/R+ intermediate risk, D–/R– the lowest risk. Other relevant risk factors for CMV infection after transplantation are the use of anti-lymphocytes antibodies as means of immunosuppression, high doses of glucocorticoids and several gene polymorphisms.

After lung transplantation, viral load testing is the cornerstone for diagnosis and monitoring for CMV infection and disease, and this can be achieved by antigenemia testing (pp65) or a quantitative PCR-based assay; the latter is currently the most widely used method because of better precision, broader linear range, faster turnaround time, higher throughput, and less risk of contamination (21). The diagnosis of tissue-invasive CMV disease should be confirmed by immunohistochemistry or *in situ* DNA hybridization. Serology should not be used to diagnose active CMV infection or disease (20).

Monitoring CMV load is also a useful approach for assessing the likelihood of drug resistance, which should be suspected in case of rising or persistently elevated viral load regardless of ongoing antiviral therapy. Resistance testing is currently performed by means of genotypic assays directly from clinical specimens: the most common mutations affect UL97 phosphotransferase and confer resistance to ganciclovir (22); UL54 DNA polymerase mutations may occur as second-step mutations in patients who already have a UL97 mutation, causing different combinations of resistance to ganciclovir, foscarnet, and/or cidofovir (23).

Recent studies focused on CMV-specific cellular immunity, which plays a crucial role in containing viral replication and can be evaluated performing QuantiFERON-CMV assay on plasma by stimulation of CD8+ T-cell responses and enzyme-linked immunosorbent spot (ELISPOT) assay on whole blood by stimulation of CD4+ and CD8+ T-cell responses; other test available include major histocompatibility complex multimer staining assays and intracellular cytokine staining assays (20).

Prevention

With regard to CMV prevention, two strategies have been used:

❖ Universal prophylaxis of recipients at high/medium risk for infection (i.e., all but D–/R–), usually with oral valganciclovir;

❖ Pre-emptive treatment of recipients with infection in order to abort the development of disease, meaning that oral valganciclovir is initiated when viral replication has reached a certain threshold.

Currently available guidelines endorse antiviral prophylaxis against pre-emptive approach based on published evidence on the safety and efficacy of the former strategy (20,24).

The American Society of Transplantation (AST) guidelines recommend 12 months of prophylaxis among CMV D+/R– lung transplant recipients and 6 to 12 months for CMV D+/R+ and D–/R+ lung transplant recipients based on the patient's risk of reactivation, drug toxicity and viral load monitoring. Prophylactic treatment should start with intravenous ganciclovir 5 mg/kg once daily, with dose adjustment for renal insufficiency; once the patient is absorbing oral medications, it can be switched to oral valganciclovir 900 mg once daily, with dose adjustment for renal insufficiency. Individuals receiving anti-lymphocyte antibodies or pulse steroids should receive CMV prophylaxis for at least 1 to 3 months after this antirejection regimen is completed. Finally, CMV immune globulin may be used as an adjunct to conventional antiviral agents in high-risk patients, but should not be used alone (25).

A pre-emptive strategy may be useful after the prophylaxis has ended, relying on constant viral load monitoring. Unfortunately, no effective vaccination is yet available for CMV.

Treatment

The approach to treatment of active disease is based

on intravenous ganciclovir and oral valganciclovir; it is important to give appropriate doses, since both these drugs should be adjusted for renal function but inadequate dosing may reduce efficacy and lead to resistance (20); their most common and significant adverse effect is myelotoxicity, in particular leuko-neutropenia and thrombocytopenia.

Valganciclovir has been showed non-inferior to intravenous ganciclovir in non-life threating CMV disease (26) and therefore should be the regimen of choice (900 mg twice daily) in these cases. However, in severe and/or invasive disease, IV ganciclovir (5 mg/kg every 12 hours) is preferable; the efficacy of antiviral treatment can be augmented by reducing the intensity of immunosuppression (21) and/or, possibly, with administration of CMV immune globulin (limited evidence).

Treatment should be administered until resolution of symptoms or viremia (two consecutive negative serum CMV load 1 week apart) occurs and/or for a minimum of 2 weeks; it should be followed by secondary suppression with oral valganciclovir 900 mg once daily for 1 to 3 months.

In case of documented ganciclovir resistance, alternatives may be IV foscarnet or cidofovir, even if the use of the latter is limited by poor clinical experience and potential severe nephrotoxicity.

Community acquired respiratory viruses (CARV)

CARV infections are of concern in lung transplantation both in terms of associated morbidity and mortality (27), and for the potential subsequent increase risk of acute and chronic lung allograft dysfunction.

The viral agents classifying as CARV generally include the following: influenza, parainfluenza (PIV), human rhinovirus (HRV), adenovirus (ADV), respiratory syncytial virus (RSV), and coronaviruses (COVs), but may be extended to other more recently identified agents such as human metapneumovirus (hMPV) and bocavirus (BCV).

The reported incidence of CARV infections among lung transplant recipients is very diverse and ranges between 7.7% and 64% (28-30). The reasons for this large variation in reported incidence are largely dependent on the applied diagnostic techniques and on seasonality issues (particularly for influenza and RSV). Studies based on serology or viral cultures alone tend to report lower detection rates whereas the more recent application of molecular biology techniques has largely expanded the diagnostic identification of these viruses. Several rapid diagnostic tools [PCR assays for serum, swab, bronchoalveolar lavage (BAL) and other

fluids] are currently available to help diagnosis and prompt management. A further development is the availability of multiplex PCR assays, that allow testing for a panel of viruses in a single determination (31).

Treatment

For a large number of viruses there is no currently available effective pharmacological treatment and management is largely supportive. For those viruses for which treatment options are available, it has shown that timely initiation is essential in order to limit complications such as ICU admission and death (32). The development of neuraminidase inhibitors (oseltamivir and zanamivir) has provided a major breakthrough in the treatment and prevention of influenza infection. These agents have largely supplanted previous drugs such as the M2 inhibitor amantadine towards which the vast majority of influenza strains (up to 90%) have developed resistance. Ribavirin is a synthetic nucleoside analogue that has been used in the treatment of different viral agents of the paramyxovirus family. It is generally administrated intravenously or in nebulised form. *Table 1* summarises available therapeutic options for CARV infections. The possible therapeutic role of steroids in the context of viral infections still remains to be completely understood.

Different studies have brought evidence both for and against the association between CARV infections and acute or chronic rejection in lung transplant recipients. A systemic review with pooled analysis involving 34 studies on CARV infections in lung transplant recipients failed to detect any association between viral infections and acute rejection (33). Data is as yet insufficient to accept or refute the association between CARV infections and the development of chronic rejection (34).

EBV

EBV is a gamma herpes virus (human herpes virus 4) and is the etiologic agent of infectious mononucleosis, persisting asymptomatically for life in most adults (90–95% in the world). Humans are the only known host for EBV. In the general population EBV is transmitted by exposure to infected body fluids such as during coughing or sneezing, or by sharing drinking or eating utensils. Following solid organ transplantation, EBV transmission from a seropositive donor to a young seronegative recipient is an important source of infection. Recent evidence indicates

Table 1 Available treatment options in community acquired respiratory virus infections.

Virus	Available treatment
Influenza	Neuraminidase inhibitors: Oseltamivir (oral); Zanamivir (nasal, intravenous)
Parainfluenza	Ribavirin (nebulised, intravenous)
Respiratory syncytial virus	Ribavirin (nebulised, intravenous)
Human metapneumovirus	Ribavirin (nebulised, intravenous)
Human rhinovirus	Supportive treatment
Adenovirus	Supportive treatment
Coronavirus	Supportive treatment
Bocavirus	Supportive treatment

that approximately 10% of lung transplant recipients present EBV mismatch (D+/R–) (35).

Acute EBV infection causes a polyclonal expansion of B cells hosting the virus and viral antigens expressed by these B cells elicit a T cell response against the majority of the infected B cells. However, a small proportion of infected B cell may escape immune surveillance; even if reactivation is not a prominent issue, in lung and other solid organ transplant recipients, when T cell immunity is waned by immunosuppressive regimen, these latently infected B cells can initiate posttransplant lymphoproliferative disorders (PTLD) (36). Recent studies suggest routine monitoring of EBV viral load by PCR on blood specimens from transplanted patients to detect possible PTLD at an early stage (37). Younger patients, particularly if EBV seronegative prior to lung transplantation are at particular risk for developing post-transplant PTLD. The rate of PTLD in lung transplant recipients may range between 5% and 15% (38).

Some transplant centers apply prophylactic antiviral treatment consisting of acyclovir or ganciclovir in high risk patients for primary EBV infection following surgery (EBV donor+, recipient EBV–), although there is no proof that this strategy has any impact on the development of post-transplant PTLD.

VZV

VZV is an exclusively human virus that is acquired either through direct contact with a skin lesion of an infected person or through airborne spread of respiratory droplets through coughing or sneezing. The vast majority of adults are seropositive having acquired the infection during infancy or adolescence, although a growing number of young adults derived VZV seropositivity from vaccination during childhood. First contact with VZV leads to acute varicella also known as chickenpox. This condition presents with systemic symptoms such as fever and malaise in addition to a diffuse vesicular, pruritic skin rash. Following initial infection, VZV is embedded in the cranial nerves and the dorsal root ganglia, establishing lifelong latency. Reactivation is possible years to decades after the initial infection in the form of herpes zoster (HZ), a flare of vesicular lesions with dermatomeric distribution associated with often intractable neuritic pain (39).

Lung transplant recipients are at increased risk for severe VZV related complications, including cutaneous dissemination and visceral end organ involvement (pneumonia, hepatitis, encephalitis), leading to a life-threatening condition (40). Reactivation of VZV typically occurs later than CMV or HSV and cutaneous lesions may be delayed or atypical with haemorrhage. Given the severity of VZV disease following transplantation, pre-transplant evaluation of recipient VZV immune status is highly advisable. It is suggested that non-immune recipients undergo VZV vaccination prior to transplantation. As for all live-attenuated vaccines, VZV vaccination post-transplantation is discouraged.

Treatment

Treatment of both post-transplant primary VZV infection and HZ reactivation should be managed with currently available antiviral agents. Localised HZ reactivation is generally treated with oral preparations and managed outside the hospital, whereas both disseminated HZ

Table 2 Treatment regimens for herpes zoster virus primary infection or reactivation following transplantation

Condition	Treatment
Local Herpes zoster (dermatomal)	Acyclovir 800 mg 5 times daily, oral or valacyclovir 1 g 3 times daily, oral
Disseminated herpes zoster	Acyclovir 10 mg/kg 3 times daily, intravenous
Acute varicella	Acyclovir 10 mg/kg 3 times daily, intravenous

reactivation and primary NZV infection require intravenous treatment and hospitalisation. *Table 2* summarises proposed treatment regimens for VZV infection or reactivation following lung transplantation. Antiviral treatment is usually prolonged for 7 days or until crust formation on skin lesions.

Prevention

Following lung transplantation, acyclovir prophylaxis to minimise the risk of HZ reactivation is generally unnecessary, as concomitant administration of CMV prophylactic agents (for example valganciclovir) is likely to prevent varicella reactivation. However, with the growing diffusion of Interferon gamma-based assays (for example CMV quantiFERON) as a basis for tailored or preemptive CMV prophylaxis, it is likely that the number of transplant recipients receiving such coverage will diminish. There is as yet no demonstration that administration of oral acyclovir in this setting may be useful to prevent HZ reactivation, although this strategy has been shown useful in other immunosuppressed populations (41). Conversely, in HZV seronegative lung transplant recipients who become exposed to an acute varicella patient, prophylaxis is recommended, given the high risk of developing a potentially life-threatening primary infection. Drugs of choice are acyclovir or valacyclovir for 7 days, beginning roughly a week after varicella exposure.

Fungi

Despite the widespread use of antimold prophylaxis, fungal infections are a frequent complication in lung transplant recipients (15–35% of patients receiving lung allografts) (42), with high morbidity, mortality and a possible role in the development of chronic lung allograft

dysfunction (43). The predominant pathogens are Aspergillus spp., Candida species, Cryptococcus spp., the agents of mucormycosis, endemic fungi (Histoplasma, Coccidioides, and Blastomyces spp.), Scedosporium spp. and Fusarium.

Aspergillus spp.

Aspergillus is the most common cause of fungal infection in lung transplant recipients (44); different species have been associated to disease in this set of patients: firstly, *A. fumigatus*; then, *A. flavus*, *A. niger* and *A. terreus* (45). Aspergilli are ubiquitous in the environment and are acquired by inhalation.

Pulmonary invasive aspergillosis is defined by invasion of lung parenchyma; it generally presents with non-specific symptoms (pleuritic chest pain, cough, fever, dyspnoea, and haemoptysis) and its development may be enhanced by several risk factors, such as allograft dysfunction, immunosuppression levels, single lung transplant, environmental exposure, colonisation (both pre- and post-transplant), hypogammaglobulinemia and CMV infection. Mortality is constantly decreasing thanks to novel antifungal treatment (45). Disseminated disease (with extension to central nervous system, joints, bone, skin, and eye) is becoming increasingly rare.

Aspergillus tracheobronchitis is seen only in lung transplant recipients and is the most common form of aspergillus infection after this transplant; it usually affects the bronchial anastomosis (with possible stenosis, dehiscence and bleeding) (46), causing productive cough, dyspnoea, fever, stridor/wheeze, haemoptysis, or acute respiratory distress.

Finally, Aspergillus colonisation is defined by lack of probable or proven Aspergillus disease; however, it has been proven a risk factor for acute and chronic rejection and for progression to invasive disease (47).

Surveillance by chest CT scan and bronchoscopy is highly recommended in lung transplant recipients, not just to screen for rejection but also to provide an extensive microbiologic sampling of the graft.

Definitive diagnosis of invasive pulmonary aspergillosis requires a biopsy demonstrating tissue invasion, but evidence of a mold in the airway is suggestive of invasive infection; CT scan findings may vary from consolidation to cavitary lesions or nodular or mass-like lesions. Detection of galactomannan antigen may help to increase sensitivity, but has a very low specificity for invasive disease (48). A

novel and promising diagnostic technique may be based on an Aspergillus PCR assay, which is currently under investigation.

Tracheobronchial aspergillosis requires systemic therapy with voriconazole in combination with nebulized amphotericin B for at least 3 months; debridement of the bronchial anastomosis may be indicated in case of abundant necrotic tissue. Voriconazole is the treatment of choice for invasive aspergillosis as well; in case of severe disease, the Infectious Diseases Society of American (IDSA) guidelines recommend combination therapy with an echinocandin (48). Like other azoles, voriconazole has significant interactions with calcineurin inhibitors and m-TOR inhibitors, which should be constantly monitored for dose reduction based on their serum levels.

Mucormycosis

The agents of Mucormycosis, which account approximately for 2% of all invasive fungal infections in transplant recipients, include the following: Rhizopus, Mucor, Rhizomucor, Lichtheimia, Cunninghamella, Apophysomyces, and Saksenaea (49). Risk factors are diabetes, renal impairment and recent rejection.

Mucormycosis is characterized by infarction and necrosis of host tissues that result from invasion of the vasculature by fungal hyphae. Pulmonary disease (consolidation/mass lesion, nodules and cavities found on the chest CT scan) is the most frequent presentation in lung transplant recipients, even if sino-orbital, cutaneous, and disseminated disease have also been reported (50). Diagnosis requires both histopathology and culture.

The overall mortality is very high, ranging from 49% to 90%. Management of mucormycosis can be very challenging: intravenous administration of lipid formulation of amphotericin B is the cornerstone of therapy in these cases, together with the reduction of immunosuppression and consideration for surgical debridement. Once the patient has stabilized, therapy may be switched to oral posaconazole (51).

Scedosporium spp.

Scedosporium spp. is a hyaline mold found in the environment, with two species capable of causing disease in humans: *S. apiospermum* and *S. prolificans*. The most common manifestations are invasive pulmonary infection and disseminated disease. Due to the intrinsic resistance to amphotericin B, voriconazole is the drug of choice in these cases, even if surgical resection may often be necessary (52).

Other

Fusarium is ubiquitous and is a notorious plant pathogen. *F. solani* and *F. oxysporum* are the most common human pathogenic species, but fusariosis is quite uncommon in lung transplant recipients, causing primarily lung involvement with or without disseminated disease (53). Fusarium is often resistant to azoles and therefore difficult to treat; amphotericin B may be an effective choice together with reducing immunosuppression.

Mycobacteria

Mycobacterial infections must be considered amongst the differential diagnoses of a lung transplant recipient presenting with infection. Both infection with *Mycobacterium tuberculosis (MTB)* and non-tuberculous mycobacteria (NTM) may play a role in the setting of lung transplantation. Common to all mycobacterial infections are the problems and delays in diagnosis, due to their fastidious culture requirements, and the complexity of treatment regimens, particularly in the current context of increasing antimicrobial resistance.

MTB

TB is still a major health threat to mankind being associated with over eight million TB-associated deaths per year, and over 2 billion people harboring latent tubercular infection worldwide (54).

Solid organ transplants recipients overall are at an increased risk of post-transplant TB compared to the general population (55). This risk is highest in the subgroup of patients undergoing lung transplantation, with reported incidence rates ranging from 6.4% to 10% (55-57). Differences in prevalence rates are partly related to the underlying degree of TB diffusion in the reporting country. Over 90% of TB cases develop within the first year following transplantation, and roughly three quarters involve the lungs (56). Crude mortality rates for post-transplant TB are in the order of 20–30%, with an attributable mortality of 10% (56,58).

Lung transplant recipients are at risk for development of TB through a number of ways. These include (in decreasing order of frequency): immunosuppression-induced

reactivation of latent TB in the recipient, acquired infection by transmission of MTB from a contagious person, or acquired from the donor during transplant. Clinical presentation of active TB generally involves systemic signs and symptoms in conjunction with respiratory symptoms, as the lung is by far the most commonly involved site, as previously mentioned. Diagnosis is hampered by heavy reliance on traditional time-consuming microbiological procedures such as culture, although the introduction of nucleic amplification tests can help both in obtaining rapid results and in differentiating MTB from NTM species.

Treatment

Treatment regimens for lung transplant recipients that develop active TB are based on the same drugs employed for MTB infection in the immunocompetent host. The two cornerstones of treatment are: a multidrug regimen in order to avoid the development of resistance, and prolonged treatment in order to achieve bacterial eradication. Treatment involves two phases: an intensive phase consisting of four drugs to be continued for 2 months or until antibiotic sensitivity testing is obtained, and a continuation phase so as to complete 6 months of treatment (59). The most effective regimen requires daily dosing throughout the entire treatment course; although alternative regimens including combinations with twice weekly or 3 times weekly drug intake are acceptable, though carry a lesser degree of efficacy. Depending on the degree of immunosuppression, prolongation of the aggressive phase to 4 months, and/or extending the continuation phase to a total of 9–12 months may be considered on an individual basis, particularly in the presence of additional risk factors such as cavitation on presenting radiograph and persisting culture positivity after 2 months of treatment (59,60). Furthermore, reduction of immunosuppressive treatment is often suggested, particularly during the early phases of treatment. *Table 3* summarizes the currently proposed treatment regimens for drug susceptible TB.

Given the number of drugs used, and the length of treatment for active TB, discontinuations due to drug induced toxicity are common even in the immunocompetent host. All the more so, following transplantation there is a considerable risk of significant drug interactions between antitubercular agents and immunosuppressants and of drug toxicity. Regarding the former, rifampin in particular induces hepatic enzymatic activity that promotes more rapid metabolism of immunosuppressive drugs and is potentially associated with increased risk of acute rejection

(61,62). Considerable increases in the doses of calcineurin inhibitors may be required (up to 3–5 fold) and in certain occasions 3 times daily administration may be preferable to twice daily calcineurin inhibitor administration. Particularly during the 4-drug aggressive phase, drug toxicity (primarily drug-induced liver injury) may be an important issue, particularly considering potential concomitant use of other hepatotoxic drugs (azathioprine, azoles, etc.). Indications for treatment discontinuation are >3-fold increase in alanine aminotransferase (ALT) levels in the presence of symptoms (nausea, vomiting, and abdominal pain), or >5-fold increase in ALT in the absence of symptoms. Drug reintroduction procedures once liver enzymes are back to baseline levels are as yet insufficiently standardized.

With the antitubercular drugs currently available, successful treatment of active TB caused by drug susceptible strains may be observed in the vast majority of patients, even after organ transplantation. Nonetheless, there is growing concern regarding the emergence of drug resistance among TB strains across the world. Multidrug-resistant (MDR) TB is defined as resistance to both isoniazid and rifampin, whereas extremely drug-resistant (XDR) TB is defined as resistance to isoniazid, rifampin, fluoroquinolones and at least one injectable drug (i.e., amikacin, kanamycin and capreomycin) (63). There is considerable country-to-country variation in the rates of both MDR and XDR, with eastern European countries showing alarmingly high rates of both forms of resistance. There is limited consensus on the optimal management of drug resistance TB in immunocompetent hosts, and only a limited number of such MDR or XDR cases have been reported following lung transplantation. General indications include using four to six drugs for the aggressive phase, involving injectable antimicrobials such as streptomycin, amikacin, kanamycin or capreomycin, and linezolid or other second-line drugs (64). It is suggested that treatment should be prolonged for up to two years following culture conversion, and is best managed with the assistance of specialists with expertise in the treatment of drug resistant TB.

Nontuberculous mycobacteria (NTM)

NTM are an ever-growing family of currently over 140 organisms that play an increasing role in pre- and post-transplant troublesome infections. NTM are free-living saprophytic organisms ubiquitous in the environment, being commonly found in soil, water supplies, dust, and plant material. Based on growth rate in culture they

Table 3 Treatment regimens for drug-susceptible tuberculosis

Drug	Induction phase		Continuation regimen	
	Dosing	Scheme	Dosing	Scheme
INH	5 mg/kg (gen. 300 mg)	Daily dose for 8 weeks	5 mg/kg (gen. 300 mg)	Daily dose for 18 weeks
RIF	10 mg/kg (gen. 600 mg)		10 mg/kg (gen. 600 mg)	
PZA	40–55 kg, 1,000 mg; 56–75 kg, 1,500 mg; 76–90 kg, 2,000 mg		–	
ETB	40–55 kg, 800 mg; 56–75 kg, 1,200 mg; 76–90 kg, 1,600 mg		–	
INH	5 mg/kg (gen. 300 mg)	Daily dose for 8 weeks	15 mg/kg (gen. 900 mg)	3 times weekly for 18 weeks
RIF	10 mg/kg (gen. 600 mg)		10 mg/kg (gen. 600 mg)	
PZA	40–55 kg, 1,000 mg; 56–75 kg, 1,500 mg; 76–90 kg, 2,000 mg		–	
ETB	40–55 kg, 800 mg; 56–75 kg, 1,200 mg; 76–90 kg, 1,600 mg		–	
INH	15 mg/kg (gen. 900 mg)	3 times weekly for 8 weeks	15 mg/kg (gen. 900 mg)	3 times weekly for 18 weeks
RIF	10 mg/kg (gen. 600 mg)		10 mg/kg (gen. 600 mg)	
PZA	40–55 kg, 1,500 mg; 56–75 kg, 2,500 mg; 76–90 kg, 3,000 mg		–	
ETB	40–55 kg, 1,500 mg; 56–75 kg, 2,500 mg; 76–90 kg, 3,000 mg		–	
INH	Daily see above: 15 mg/kg (gen. 900 mg)	Daily for 2 weeks then twice weekly for 6 weeks	15 mg/kg (gen. 900 mg)	Twice weekly for 18 weeks
RIF	Daily see above: 10 mg/kg (gen. 600 mg)		10 mg/kg (gen. 600 mg)	
PZA	Daily see above: 40–55 kg, 2,000 mg; 56–75 kg, 3,000 mg; 76–90 kg, 4,000 mg		–	
ETB	Daily see above: 40–55 kg, 2,000 mg; 56–75 kg, 2,800 mg; 76–90 kg, 4,000 mg		–	

INH, isoniazid; RIF, rifampin; PZA, pyrazinamide; ETB, ethambutol; gen. generally.

are usually classified as rapid growers, which generally form visible colonies in culture within a week, and slow growers that behave more like MTB in culture. *Table 4* lists currently known MTB associated with disease in lung transplant recipients. Contrary to MTB, there is no human-to-human transmission of NTM, at least in the immunocompetent host, whereas this may only be partially true in the presence of structural lung damage or immunosuppression (65).

Several disease conditions that may eventually require lung transplantation develop pulmonary structural derangements associated with acquisition of NTM infection prior to transplantation. These include, COPD,

pneumoconiosis and cystic fibrosis. Currently, roughly 12% of cystic fibrosis patients present a positive NTM culture prior to transplantation (61). Reported overall incidence of NTM infection among lung transplant recipients varies between 3.8% to 22.4%, but not all cases necessarily require treatment (66,67). Infection generally occurs at a considerable time distance from transplantation (averaging 2 years) indicating that most infections are acquired following transplantation, although persistence of NTM harbored prior to transplantation is an issue in cystic fibrosis patients, and in COPD patients who undergo single lung transplantation. It is thought that transplant recipients may be at higher risk of acquiring nosocomial NTM infections

Table 4 Nontuberculous mycobacteria associated with infection in lung transplant recipients

Fast growers
M. abscessus
M. bolletii
M. chelonae
M. fortuitum
M. margeritense
M. massiliense
M. mucogenicum
M. neoaurum
Slow growers
M. asiaticum
M. avium
M. celatum
M. genavense
M. haemophilum
M. intracellulare
M. gastri
M. gordonae
M. kansasii
M. malmoense
M. marinum
M. scrofulaceum
M. szulgai
M. terrae
M. thermoresistibile
M. triplex
M. xenopi

from contaminated water supplies (68).

NTM infections in solid organ transplant recipients as a class may develop as disseminated disease, pulmonary infection, skin and soft tissues infection, musculoskeletal infections, or catheter-related infection. Conversely, following lung transplantation pulmonary involvement is more common, but not exclusive. Additionally, lung transplant recipients may develop both surgical wound and bronchial and vascular anastomotic NTM infections in the early perioperative period, particularly with *M. abscessus*. Pulmonary involvement may present as nodules, pulmonary infiltrates, abscesses and cavitating nodules. Systemic symptoms such as fever are not universally present, whereas respiratory symptoms may include chronic cough, sputum production, dyspnea and, occasionally, hemoptysis (69). Diagnosis is based on typical imaging findings for pulmonary NTM, in addition to positive cultures, or biopsy of involved tissues (e.g., skin).

Pre-transplant culturing of *M. abscessus*, an increasingly common event in cystic fibrosis patients, has become a growing concern in deciding eligibility for transplantation, based both on the bacterium's intrinsic resistance to most antibiotics, and the risk of disseminated uncontrolled infection post-surgery. Whether *M. abscessus* infection negatively impacts on lung transplant survival is still a matter of debate. The latest international consensus on the indications for lung transplantation suggests that presence of NTM infection prior to surgery, including *M. abscessus*, may be considered a contraindication for transplantation if there is progressive disease despite optimal therapy, or when such treatment is not tolerated due to side effects (70). More properly we should now refer to the *M. abscessus* complex (MABSC), which includes three closely related species of NTM: *M. abscessus* sensu stricto (referred to as *M. abscessus*), *M. massiliense*, and *M. bolletii* (71). The latter is relatively uncommon accounting for roughly 10% of isolates, whereas *M. abscessus* and *M. massilense* make up the remaining 90% of isolates and are fairly evenly distributed between them. When MABSC is cultured in a potential lung transplant candidate, genotyping is important in order to distinguish between species. In fact, *M. massiliense* generally presents a lesser degree of antimicrobial resistance compared to *M. abscessus* due to the absence of the erythromycin ribosomal methylase gene, erm (41), which encodes for inducible clarithromycin resistance (72). In addition, pre-transplant infection with *M. massiliense* is more likely to clear following transplantation compared to *M. abscessus* (73). Microbiological differentiation between species is therefore important in considering eligibility for lung transplantation.

Treatment

Treatment of NTM infection is based on the same basic principles that guide management of MTB infection: a multiple drug regimen in order to reduce the risk of developing resistance, and prolonged treatment in order to obtain bacterial eradication. It has been suggested that

Table 5 Treatment regimens for slow growing nontuberculous mycobacteriosis

Pathogen	Recommended regimen	Second-line or additional agents
M. avium complex	Azithromycin 250–500 mg daily	Clarithromycin 500 mg twice daily
	Rifabutin 300 mg daily	Rifampin 10 mg/kg daily (max. 600 mg)
	Ethambutol 15 mg/kg daily	Amikacin 10–30 mg/kg once daily or Streptomycin 15 mg/kg daily (max. 1,000 mg)
M. kansasii	Rifabutin 300 mg daily	Rifampin 10 mg/kg daily (max. 600 mg)
	Ethambutol 15 mg/kg daily	Clarithromycin 500 mg twice daily or azithromycin 500 mg daily
	Isoniazid 5 mg/kg daily (max. 300 mg)	Trimethoprim-sulfamethoxazole 960 mg twice daily
		Moxifloxacin 400 mg daily
		Amikacin 10–30 mg/kg once daily or streptomycin 15 mg/kg daily (max. 1,000 mg)
M. marinum	Azithromycin 250–500 mg daily	Rifampin 10 mg/kg daily (max. 600 mg)
	Ethambutol 15 mg/kg daily (consider adding rifabutin 300 mg daily for extensive disease)	Clarithromycin 500 mg twice daily or azithromycin 250–500 mg daily
		Trimethoprim-sulfamethoxazole 960 mg twice daily
		Doxycycline or minocycline 100 mg twice daily
M. haemophilum	Azithromycin 500 mg daily	Rifampin 10 mg/kg daily (max. 600 mg)
	Rifabutin 300 mg daily	Clarithromycin 500 mg twice daily or azithromycin 500 mg daily
	Ciprofloxacin 500 mg twice daily	Trimethoprim-sulfamethoxazole 960 mg twice daily
		Doxycycline 100 mg twice daily

treatment of MABSC should involve an intensive phase followed by a continuation phase, similarly to MTB infection, although duration of the intensive phase is as yet undefined and based on the severity of infection (74). *Tables 5,6* summarize indications for treatment regimens for slow growing and fast growing NTM, respectively (9,74). Optimal duration of treatment, particularly in the setting of lung transplantation is as yet undetermined. In some skin and soft tissue infections 6 months of treatment may be sufficient, whereas in pulmonary and disseminated disease treatment should be prescribed for 12 months following culture negativity. Drug toxicity may be a significant problem due to the multi drug treatment regimens and length of treatment. Periodic evaluation for potential drug toxicities (hearing loss, visual loss, renal impairment and liver function tests) should be performed throughout treatment. In adjunction to medical therapy, surgical excision and reduction of immunosuppressive levels may be considered.

Nocardia

Nocardia is saprophytic gram-positive aerobic actinomycetes, ubiquitous in the environment (75). Over 80 Nocardia species have currently been identified, of which 30 have been associated with disease in humans.

Nocardia infections may cause both localized and systemic suppurative disease (76); it can disseminate to any organ (in particular the central nervous system) and tends to relapse or progress despite therapy. It can be acquired by inhalation, direct inoculation and/or ingestion. Nocardiosis is typically considered an opportunistic infection and its risk is highest in the first year following organ transplantation, especially for lung recipients. Recent data indicate a 3.5% rate of Nocardia infections amongst lung transplant recipients (77). *N. nova* and *N. farcinica* generally account for more than 75% of the infections and the majority of patients are affected by pulmonary disease. Other commonly encountered nocardia species in the

Table 6 Proposed treatment regimens for rapidly growing NTM

Pathogen	Recommended regimen	Second-line or additional agents
M. *abscessus*	Azithromycin 250–500 mg once daily; plus amikacin 15 mg/kg daily, imipenem 1 g twice daily, or cefoxitin 70 mg/kg 3 times a day; or two parenteral agents	Clarithromycin 500 mg twice daily
		Linezolid 600 mg once or twice daily
		Tigecycline 100 mg loading dose then 50 mg once or twice daily
M. *chelonae*	Two drugs: Azithromycin 250–500 mg daily; plus amikacin 15 mg/kg once daily, or tobramycin 5 mg/kg once daily, linezolid 600 mg once or twice daily, tigecycline 100 mg loading dose then 50 mg once or twice daily, or imipenem 1 g twice daily	According to sensibility testing
M. *fortuitum*	Two drugs: amikacin 15 mg/kg daily; ciprofloxacin 500 mg twice daily or other quinolones; trimethoprim-sulfamethoxazole 960 mg twice daily; cefoxitin 70 mg/kg 3 times a day; clarithromycin 500 mg twice daily	Azithromycin 250–500 mg daily
		Trimethoprim-sulfamethoxazole 960 mg twice daily
		Doxycycline or minocycline 100 mg twice daily
		Imipenem 1 g twice daily
		Tigecycline 100 mg loading dose then 50 mg once or twice daily
		Moxifloxacin 400 mg daily

NTM, non-tuberculous mycobacteria.

setting of solid organ transplantation include *N. asteroides* sensu stricto, *N. brasiliensis*, *N. otitidiscaviarum*, and *N. transvalensis*. Hospital outbreaks have occurred among transplant recipients, diffusion being likely mediated by healthcare worker hands, air contamination, or vicinity with construction sites. Nocardia infection should be suspected particularly in febrile events with pulmonary or extrapulmonary involvement that initially respond to antibiotic treatment but relapse on termination of standard duration schemes. Whenever such an infection is suspected, special staining (modified acid-fast bacillus) and cultures should be promptly requested.

Treatment

Treatment of nocardial infections following lung transplantation is often based on a combination of more than one antimicrobial agent, given the risk of antimicrobial resistance, and the high associated morbidity and mortality in this setting (78). *Table* 7 provides guidance for antimicrobial choices in treating nocardial infections. Individualised treatment is however common based on antimicrobial sensibility testing and the development of drug toxicity may dictate antimicrobial substitution during therapy. Prolonged treatment is usually required, although optimal duration is as yet undefined. Standard regimens

in immunocompromised hosts suggest 6–12 months of treatment, although shorter courses have been successful, provided there be an initial phase of parenteral antibiotic management. In presence of nocardial soft tissue abscesses, surgical drainage and reduction in immunosuppression may be useful adjuncts to antimicrobial therapy.

Prevention

Most lung transplant recipients receive prolonged *Pneumocystis jirovecii* prophylaxis with co-trimoxazole. Given the activity of this agent towards Nocardia species, it may be reasonable to assume that this would provide sufficient coverage to prevent the development of nocardia infections. Published case series however indicate that most lung transplant patients who develop nocardia infections do so while receiving co-trimoxazole prophylaxis (79). Reasons for insufficient prophylactic coverage probably involve increases in antimicrobial resistance over time and the failure to obtain adequate serum levels for nocardial prevention with the 3 times weekly regimen employed for pneumocystis prevention. Increasing co-trimoxazole dosing regimen so as to provide primary prevention towards nocardia species is an as yet untested possibility that must be counterbalanced with the increased risk of drug toxicity. Similarly, long term co-trimoxazole has been used as secondary prophylaxis

Table 7 Treatment schemes for Nocardia infection following lung transplantation

Condition	Initial scheme	Alternative
Non-critical	Trimethoprim-sulfamethoxazole 15 mg/kg in 3–4 daily doses	Imipenem 500 mg 4 times daily or meropenem 1 g 3 times daily + amikacin 10–15 mg/kg once daily or minocycline 100 mg twice daily or linezolid 600 mg twice daily
Critical (disseminated, cerebral disease)	Imipenem 500 mg 4 times daily or meropenem 1 g 3 times daily + amikacin 10–15 mg/kg once daily or trimethoprim-sulfamethoxazole 15 mg/kg in 3–4 daily doses	Linezolid 600 mg twice daily or ceftriaxone 2 g twice daily or cefotaxime 2 g 3 times daily or minocycline 100 mg twice daily

following nocardia infection (79), although there is no consensus on optimal dosing regimens.

Clostridium difficile (C. difficile)

Clostridium difficile (*C. difficile*) is a spore-forming, anaerobic, Gram positive bacillus. In the general population it is reported to cause 6–25% of cases of antibiotic associated diarrhea, over 70% of antibiotic-associated colitis, and over 90% of cases of antibiotic-associated pseudomembranous colitis (80). Intestinal tissue damage is mediated through toxin A and toxin B, which trigger a cytotoxic response, neutrophilic infiltrate and cytokine release.

C. difficile infection is a common problem in lung transplant recipients, with an estimated incidence of 7–31% (81). Several risk factors have been identified: prolonged hospitalization and ICU stay, intense immunosuppression, exposure to antimicrobial agents with gram positive activity. Presentation is often atypical, with little diarrhoea; abdomen CT scan may be useful to rule out severe disease (pseudomembranous colitis), which is associated with a high risk of bowel perforation.

CDI is diagnosed by confirming the presence of toxigenic *C. difficile* in the stool of a symptomatic patient. Indications for treatment options based on severity of disease have recently been issued for organ transplant recipients (82). In the absence of complications such as ileus, toxic megacolon or multiorgan failure, oral metronidazole 500 mg 3 times daily for 10–14 days is the initial treatment of choice. Conversely, in presence of the above complications, combination treatment with intravenous metronidazole and oral vancomycin 500 mg 4 times daily should be initiated. In uncomplicated cases started on oral metronidazole, failure to respond within 5 days should prompt switch to oral vancomycin 125 mg 4 times

daily. Once treatment has been discontinued, should symptoms relapse, retreatment may be initiated with either metronidazole or vancomycin, based on the severity of symptoms (82).

Pneumocystis jirovecii pneumonia

Pneumocystis jirovecii (previously *Pneumocystis carinii*), is a component of the Taphrinomycotina branch of the fungal kingdom. *Pneumocystis* spp. are ubiquitous in nature, and apparently infect children in early life with probable airborne person to person transmission (83). Immunocompetent persons usually clear the infection in the absence of any symptoms; but in transplanted patients PCP progresses to severe inflammatory pneumonia with respiratory failure and death. In these immunocompromised patients, PCP has a mortality rate ranging between 20% and 40%, which is the double that reported for HIV patients (84). In the past PCP rates among solid organ transplant recipients were in the order of 5–15%, and up to 20–40% in lung transplant recipients. However, the widespread use of sulfamethoxazole-trimethoprim prophylaxis has now dramatically reduced infection rates to 0.3–2.6% (85). Prior to prophylaxis, risk of PCP was highest during the first 6 months following transplantation, thus leading to the recommendation to sustain prophylaxis for 6–12 months post-surgery (86).

Clinical presentation of PCP includes fever, dry cough, dyspnea and chest pain, night sweats and weight loss. Pneumothorax is a possible complication. Patients generally present marked hypoxemia, out of proportion compared to physical findings. Symptoms may rapidly evolve in 1–2 days, or sometimes be more slowly protracted over 7–10 days. Initial chest X-ray may be unremarkable in about 30% of patients. if there are signs, these typically include

Table 8 Treatment options for *Pneumocystis* pneumonia

Agent	Dose
Trimethoprim-Sulfamethoxazole	15–20 mg/kg in 3–4 daily doses
Pentamidine	4 mg/kg daily
Atovaquone	750 mg twice daily
Primaquine and clindamycin	Primaquine 15–30 mg daily with clindamycin 600–900 mg 3–4 times daily
Dapsone and trimethoprim	Dapsone 100 mg daily with trimethoprim 15 mg/kg in 3 daily doses

bilateral diffuse symmetric finely granular infiltrates with characteristic central location and fast progression to the peripheral parts of the lungs. Lung nodules (which may cavitate) and hilar lymphadenopathy are possible findings; furthermore, upper lobes may present cysts. Chest CT may be highly suggestive presenting bilateral, asymmetric patchy mosaic appearance with ground-glass pattern, thickening of lobular septa, nodules with possible excavation, pneumatoceles, hilar lymphadenopathy, pleural effusion and pneumothorax.

High values of serum lactate dehydrogenase are a suggestive indicator of PCP in HIV-infected patients, but this does not apply in transplanted patients. Elevated serum levels of beta-D-glucan (a fungal cell-wall element) could support the diagnosis of PCP; the negative predictive value of beta-D-glucan is consistently high, even though a high rate of false positive results has been described. Immunofluorescent microscopic visualization of *Pneumocystis* in bronchoalveolar lavage is the standard method for the laboratory diagnosis of PCP (87). Unfortunately, transplant patients often develop PCP with a lower fungal burden than patients with other forms of immunodeficiency, consequently a consistent number of patients without *Pneumocystis* identification on the microscopic smear are effectively affected by PCP. PCR techniques have been developed to identify *Pneumocystis* DNA in bronchoalveolar lavage (or other biological fluid); in particular, single-copy real-time PCR may distinguish patients with infection from those with colonization (88).

Treatment

If PCP is suspected on clinical and radiological grounds and beta-D-glucan values are high, adequate therapy

initiation should not be delayed while awaiting *Pneumocystis* identification on bronchoalveolar lavage. Intravenous trimethoprim-sulfamethoxazole is the treatment of choice; it must be adjusted on renal function and generally continued for 3 weeks. In patients who are allergic to the drug or who develop toxicity during treatment, alternative antimicrobial regimens are available (89) (see *Table 8*). Corticosteroid treatment is added in hypoxemic patients (PaO$_2$ <70 mmHg on room air) as this has been shown to exert beneficial survival effects in HIV patients, although the additional benefit has never been tested in solid organ recipients. Reduction of immunosuppressive levels is generally recommended.

In the current era of 1-year PCP prophylaxis, it has been found that a considerable number of delayed onset PCP cases may develop during the second year post lung transplantation, supporting the notion that prolonged PCP prophylaxis may be necessary in lung transplant recipients (90).

Toxoplasmosis

In developed countries, *Toxoplasma gondii* is one of the most common parasites; France is particularly involved, since more than 50% of citizens are infected (91). *Toxoplasma gondii* is an obligate apicomplexan intracellular protozoan that can infect a large number of warm-blooded animals; cats are the final host, where the organisms undergo sexual reproduction determining the environmental dissemination of highly resistant oocysts with the feces. In the human body, an intermediate host, the oocysts' wall is degraded by intestinal proteolytic enzymes; then the protozoa enter the intestinal epithelium where they differentiate into tachyzoites, which represent the cellular stage of *Toxoplasma gondii*. Tachyzoites can quickly multiply and move through tissues; in no more than 10 days from ingestion, the brain and muscles are contaminated with *Toxoplasma gondii* cysts.

Immunocompetent persons present a subclinical course when infected from Toxoplasma; on the contrary, in immunocompromised individuals this parasite could develop life-threatening disease. Among transplantation procedures, heart transplantation has the greatest risk for toxoplasmosis; myocarditis generally occurs within 6 months from surgery, but brain abscess, pneumonia and disseminated infections are also possible. Serologically mismatched patients (seropositive donor/seronegative recipients) have the highest risk of developing the disease (92). Toxoplasmosis is less common in lung transplantation since anti-PCP prophylaxis is extensively applied in all centers. When

the lungs are involved, radiological signs include bilateral ground-glass infiltrates, septal and peribronchial thickening, miliary multiple nodules, lymph node enlargement and pleural effusion. The diagnosis of toxoplasmosis is typically made by serologic testing but identification of the parasite in bronchoalveolar lavage, or even histologic examination showing necrotizing inflammation and characteristic organisms are needed for definitive diagnosis.

The therapeutic regimen is bases on pyrimethamine with the addition of leucovorin calcium and sulfadiazine or clindamycin; treatment generally lasts 6 weeks.

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Footnote

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References

1. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med 2007;357:2601.

2. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.

3. Yu XY, Wang Y, Zhong H, et al. Diagnostic value of serum procalcitonin in solid organ transplant recipients: a systematic review and meta-analysis. Transplant Proc 2014;46:26-32.

4. Hemmert C, Ohana M, Jeung MY, et al. Imaging of lung transplant complications. Diagn Interv Imaging 2014;95:399-409.

5. Lehto JT, Koskinen PK, Anttila VJ, et al. Bronchoscopy in the diagnosis and surveillance of respiratory infections in lung and heart-lung transplant recipients. Transpl Int 2005;18:562-71.

6. Solís A, Brown D, Hughes J, et al. Methicillin-resistant Staphylococcus aureus in children with cystic fibrosis: An eradication protocol. Pediatr Pulmonol 2003;36:189-95.

7. Ziakas PD, Pliakos EE, Zervou FN, et al. MRSA and VRE colonization in solid organ transplantation: a meta-analysis of published studies. Am J Transplant 2014;14:1887-94.

8. Davido B, Batista R, Michelon H, et al. Is faecal microbiota transplantation an option to eradicate highly drug-resistant enteric bacteria carriage? J Hosp Infect 2017;95:433-7.

9. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007;175:367-416. Erratum in: Am J Respir Crit Care Med 2007;175:744-5.

10. Smibert O, Snell GI, Bills H, et al. Mycobacterium abscessus Complex - a Particular Challenge in the Setting of Lung Transplantation. Expert Rev Anti Infect Ther 2016;14:325-33.

11. Benden C, Danziger-Isakov LA, Astor T, et al. Variability in immunization guidelines in children before and after lung transplantation. Pediatr Transplant 2007;11:882-7.

12. Horan TC, Gaynes TP, Martone WJ, et al. CDC Definitions of Nosocomial Surgical Site Infections, 1992: A Modification of CDC Definitions of Surgical Wound Infections. Infect Control Hosp Epidemiol 1992;13:606-8.

13. Edwards FH, Engelman RM, Houck P, et al. The Society of Thoracic Surgeons Practice Guideline Series: Antibiotic Prophylaxis in Cardiac Surgery, Part I: Duration. Ann Thorac Surg 2006;81:397-404.

14. Nash EF, Coonar A, Kremer R, et al. Survival of Burkholderia cepacia sepsis following lung transplantation in recipients with cystic fibrosis. Transpl Infect Dis 2010;12:551-4.

15. Pilarczyk K, Haake N, Heckmann J, et al. Is universal antifungal prophylaxis mandatory in adults after lung transplantation? A review and meta-analysis of observational studies. Clin Transplant 2016;30:1522-31.

16. Zamora MR. Cytomegalovirus and lung transplantation. Am J Transplant 2004;4:1219.

17. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. J Heart Lung Transplant 2002;21:297.

18. Sharples LD, McNeil K, Stewart S, et al. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. J Heart Lung Transplant 2002;21:271.

19. Razonable RR, Limaye RR. Cytomegalovirus infection after solid organ transplantation. In: Bowden RA, Ljungman P, Snydman DR. editors. Transplant Infections, 3rd edition. Philadelphia: Lippincott Williams and Wilkins, 2010:328.

20. Kotton CN, Kumar D, Caliendo AM, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 2013;96:333.

21. Piiparinen H, Hockerstedt K, Gronhagen-Riska C, et al. Comparison of two quantitative CMV PCR tests, Cobas Amplicor CMV Monitor and TaqMan assay, and pp65-antigenemia assay in the determination of viral loads from peripheral blood of organ transplant patients. J Clin Virol 2004;30:258.

22. Chou S, Marousek GI, Van Wechel LC, et al. Growth and drug resistance phenotypes resulting from cytomegalovirus DNA polymerase region III mutations observed in clinical specimens. Antimicrob Agents Chemother 2007;51:4160-2.

23. Hakki M, Chou S. The biology of cytomegalovirus drug resistance. Curr Opin Infect Dis 2011;24:605-11.

24. Razonable RR, Humar A; AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplantation. Am J Transplant 2013;13 Suppl 4:93-106.

25. Valantine HA, Luikart H, Doyle R, et al. Impact of cytomegalovirus hyperimmune globulin on outcome after cardiothoracic transplantation: a comparative study of combined prophylaxis with CMV hyperimmune globulin plus ganciclovir versus ganciclovir alone. Transplantation 2001;72:1647-52.

26. Asberg A, Humar A, Rollag H, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant 2007;7:2106-13.

27. Shalhoub S, Husain S. Community-acquired respiratory viral infections in lung transplant recipients. Curr Opin Infect Dis 2013;26:302-8.

28. Gottlieb J, Schulz TF, Welte T, et al. Community-acquired respiratory viral infections in lung transplant recipients: a single season cohort study. Transplantation 2009;87:1530-7.

29. Garbino J, Soccal PM, Aubert JD, et al. Respiratory viruses in bronchoalveolar lavage: a hospital-based cohort study in adults. Thorax 2009;64:399-404.

30. Speich R, van der Bij W. Epidemiology and management of infections after lung transplantation. Clin Infect Dis 2001;33:S58-65.

31. Mahony J, Chong S, Merante F, et al. Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay. J Clin Microbiol 2007;45:2965-70.

32. Muthuri SG, Myles PR, Venkatesan S, et al. Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009–2010 influenza A(H1N1) pandemic: a systematic review and meta-analysis in hospitalized patients. J Infect Dis 2013;207:553-63.

33. Vu DL, Bridevaux PO, Aubert JD, et al. Respiratory viruses in lung transplant recipients: a critical review and pooled analysis of clinical studies. Am J Transplant 2011;11:1071-8.

34. Allyn PR, Duffy EL, Humphries RM, et al. Graft Loss and CLAD-onset is hastened by viral pneumonia after lung transplantation. Transplantation 2016;100: 2424-31.

35. Kotton CN, Huprikar S, Kumar D. Transplant Infectious Diseases: A review of the scientific registry of transplant recipients published data. Am J Transplant 2017;17:1439-46.

36. Kieff E, Rickinson AB. Epstein Barr virus and its replication. In: Knipe DM, Howley PM. editors. Fields Virology. 5th edition. Philadelphia: Lippincott Williams and Wilkins, 2007. pp2603.

37. Tsai DE, Douglas L, Andreadis C, et al. EBV PCR in the diagnosis and monitoring of post transplant lymphoproliferative disorder: results of a two-arm prospective trial. Am J Transplant 2008;8:1016-24.

38. Neuringer IP. Posttransplant lymphoproliferative disease after lung transplantation. Clin Dev Immunol 2013;2013:430209.

39. Pergam SA, Limaye AP; AST Infectious Diseases Community of Practice. Varicella zoster virus in solid organ transplantation. Am J Transplant 2013;13 Suppl 4:138-46.

40. Miller GG, Dummer JS. Herpes simplex and varicella zoster viruses: forgotten but not gone. Am J Transplant 2007;7:741-7.

41. Erard V, Guthrie KA, Varley C, et al. One-year acyclovir prophylaxis for preventing varicella-zoster virus disease after hematopoietic cell transplantation: No evidence of rebound varicella-zoster virus disease after drug discontinuation. Blood 2007;110:3071-7.

42. Christie JD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. J Heart Lung Transplant 2012;31:1073-86.

43. Husain S, Singh N. Bronchiolitis obliterans and lung transplantation: evidence for an infectious etiology. Semin Respir Infect 2002;17:310-4.

44. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis 2010;50:1101-11

45. Steinbach WJ, Marr KA, Anaissie EJ, et al. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. J Infect 2012;65:453-64.

46. Nunley DR, Gal AA, Vega JD, et al. Saprophytic fungal infections and complications involving the bronchial anastomosis following human lung transplantation. Chest 2002;122:1185-91.

47. Weigt SS, Elashoff RM, Huang C, et al. Aspergillus colonization of the lung allograft is a risk factor for bronchiolitis obliterans syndrome. Am J Transplant 2009;9:1903-11.

48. Patterson TF, Thompson GR 3rd, Denning DW, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis 2016;63:e1-60 .

49. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoramycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. Clin Infect Dis 2012;54 Suppl 1:S8-15.

50. Sun HY, Aguado JM, Bonatti H, et al; Zygomycosis Transplant Study Group. Pulmonary zygomycosis in solid organ transplant recipients in the current era. Am J Transplant 2009;9:2166-71.

51. Bhaskaran A, Hosseini-Moghaddam SM, Rotstein C, et al. Mold Infections in Lung Transplant Recipients. Semin Respir Crit Care Med 2013;34:371-9.

52. Cortez KJ, Roilides E, Quiroz-Telles F, et al. Infections caused by Scedosporium spp. Clin Microbiol Rev 2008;21:157-97.

53. Carneiro HA, Coleman JJ, Restrepo A, et al. Fusarium infection in lung transplant patients: report of 6 cases and review of the literature. Medicine (Baltimore) 2011;90:69-80.

54. Zumla A, George A, Sharma V, et al. The WHO 2014 global tuberculosis report--further to go. Lancet Glob Health 2015;3:e10-2.

55. Muñoz P, Rodriguez C, Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. Clin Infect Dis 2005;40:581-7.

56. Torre-Cisneros J, Doblas A, Aguado JM, et al. Tuberculosis after solid-organ transplant: incidence, risk factors and clinical characteristics in the RESITRA (Spanish Network of Infection in Transplantation) cohort. Clin Infect Dis 2009;48:1657-65.

57. Meije Y, Piersimoni C, Torre-Cisneros J, et al. Mycobacterial infections in solid organ transplant recipients. Clin Microbiol Infect 2014;20:89-101.

58. Holty JE, Sista RR. Mycobacterium tuberculosis infection in transplant recipients: Early diagnosis and treatment of resistant tuberculosis. Curr Opin Organ Transplant 2009;14: 613-8.

59. Nahid P, Dorman SE, Alipanah N, et al. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. Clin Infect Dis 2016;63:e147-95.

60. Aguado JM, Torre-Cisneros J, Fortun J, et al. Tuberculosis in solid-organ transplant recipients: consensus statement of the group for the study of infection in transplant recipients (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology. Clin Infect Dis 2009;48:1276-84.

61. Pena T, Klesney-Tait J. Mycobacterial infections in solid organ and hematopoietic stem cell transplantation. Clin Chest Med 2017;38:761-70.

62. Modry DL, Stinson EB, Oyer PE, et al. Acute rejection and massive cyclosporine requirements in heart transplant recipients treated with rifampin. Transplantation 1985;39:313-4.

63. Kliiman K, Altraja A. Predictors of extensively drug-resistant pulmonary tuberculosis. Ann Intern Med 2009;150:766-75.

64. Falzon D, Jaramillo E, Schunemann HJ, et al. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur Respir J 2011;38:516-28.

65. Aitken ML, Limaye A, Pottinger P, et al. Respiratory outbreak of Mycobacterium abscessus subspecies massiliense in a lung transplant and cystic fibrosis center. Am J Respir Crit Care Med 2012;185:231-2.

66. Kesten S, Chaparro C. Mycobacterial infections in lung transplant recipients. Chest 1999;115:741-5.

67. Knoll BM, Kappagoda S, Gill RR, et al. Non-tuberculous mycobacterial infection among lung transplant recipients: a 15-year cohort study. Transpl Infect Dis 2012;14:452-60.

68. Baker AW, Lewis SS, Alexander BD, et al. Two-phase hospital-associated outbreak of Mycobacterium abscessus: investigation and mitigation. Clin Infect Dis 2017;64:902-11.

69. Keating MR, Daly JS. Nontuberculous mycobacterial infections in solid organ transplantation. Am J Transplant 2013;13:77-82.

70. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.

71. Leao SC, Tortoli E, Euzéby JP, et al. Proposal that Mycobacterium massiliense and Mycobacterium bolletii

be united and reclassified as Mycobacterium abscessus subsp. bolletii comb. nov., designation of Mycobacterium abscessus subsp. abscessus subsp. nov. and emended description of Mycobacterium abscessus. Int J Syst Evol Microbiol 2011;61:2311-3.

72. Koh WJ, Jeon K, Lee NY, et al. Clinical Significance of Differentiation of Mycobacterium massiliense from Mycobacterium abscessus. Am J Respir Crit Care Med 2011;183:405-10.

73. Roux AL, Catherinot E, Soismier N, et al. Comparing Mycobacterium massiliense and Mycobacterium abscessus lung infections in cystic fibrosis patients. J Cyst Fibros 2015;14:63-9.

74. Piersimoni C. Nontuberculous mycobacteria infection in solid organ transplant recipients. Eur J Clin Microbiol Infect Dis 2012;31:397-403.

75. Lerner PI. Nocardiosis. Clin Infect Dis 1996;22:891-903.

76. Lederman ER, Crum NF. A case series and focused review of nocardiosis: clinical and microbiologic aspects. Medicine (Baltimore) 2004;83:300.

77. Peleg AY, Husain S, Qureshi ZA, et al. Risk factors, clinical characteristics, and outcome of Nocardia infection in organ transplant recipients: A matched case–control study. Clin Infect Dis 2007;44:1307-14.

78. Clark NM, Reid GE; AST Infectious Diseases Community of Practice. Nocardia infections in solid organ transplantation. Am J Transplant 2013;13 Suppl 4:83-92.

79. Poonyagariyagorn HK, Gershman A, Avery R, et al. Challenges in the diagnosis and management of Nocardia infections in lung transplant recipients. Transpl Infect Dis 2008;10:403-8.

80. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol 2010;31:431-55.

81. Paudel S, Zacharioudakis IM, Zervou FN, et al. Prevalence of Clostridium difficile infection among solid organ transplant recipients: a meta-analysis of published studies. PLoS One 2015;10:e0124483.

82. Dubberke ER, Burdette SD; AST Infectious Diseases Community of Practice. Clostridium difficile infections in solid organ transplantation. Am J Transplant 2013;13 Suppl 4:42-9.

83. Morris A, Wei K, Afshar K, et al. Epidemiology and clinical significance of pneumocystis colonization. J Infect Dis 2008;197:10-7.

84. Mansharamani NG, Garland R, Delaney D, et al. Management and outcome patterns for adult Pneumocystis carinii pneumonia, 1985 to 1995: comparison of HIV-associated cases to other immunocompromised states. Chest 2000;118:704-11.

85. Iriart X, Challan Belval T, Fillaux J, et al. Risk factors of Pneumocystis pneumonia in solid organ recipients in the era of the common use of posttransplantation prophylaxis. Am J Transplant 2015;15:190-9.

86. Martin SI, Fishman JA. Pneumocystis pneumonia in solid organ transplant recipients. Am J Transplant 2009;9:S227-33.

87. Procop GW, Haddad S, Quinn J, et al. Detection of Pneumocystis jiroveci in respiratory specimens by four staining methods. J Clin Microbiol 2004;42:3333-5.

88. Wilson JW, Limper AH, Grys TE, et al. Pneumocystis jirovecii testing by real-time polymerase chain reaction and direct examination among immunocompetent and immunosuppressed patient groups and correlation to disease specificity. Diagn Microbiol Infect Dis 2011;69:145-52.

89. Smego RA Jr, Nagar S, Maloba B, et al. A meta-analysis of salvage therapy for Pneumocystis carinii pneumonia. Arch Intern Med 2001;161:1529-33.

90. Wang EHZ, Partovi N, Levy RD, et al. Pneumocystis pneumonia in solid organ transplant recipients: not yet an infection of the past. Transpl Infect Dis 2012;14:519-25.

91. Flegr J, Prandota J, Sovičková M, et al. Toxoplasmosis-a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PLoS One 2014;24;9:e90203.

92. Derouin F, Pelloux H; ESCMID Study Group on Clinical Parasitology. Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infect 2008;14:1089-101.

Lung transplantation in elderly patients

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Abstract: Consensus statements on the selection of lung transplant candidates have consistently identified older age as a relative contraindication to transplantation. A combination of population-level demographic changes, revision of the lung allocation score (LAS), and clearer data on outcomes in elderly transplant recipients has, however, driven a steady increase in the threshold at which age is taken into consideration. This article reviews the current state of lung transplantation in elderly patients with an emphasis on the factors that have increased lung transplantation in older age groups, their expected outcomes including survival and health-related quality of life, and the factors that go in to appropriate candidate and procedure selection in this population.

Keywords: Age; elderly; lung allocation; lung transplantation

Introduction

Consensus statements on the selection of lung transplant candidates have consistently identified older age as a relative contraindication to transplantation. International guidelines in the 1990s recommended against bilateral lung transplant in individuals over 60 and against single transplant in those over 65 (1). By the mid-2000s, the guidelines were revised to state that, although there is no upper age limit representing an absolute contraindication to transplantation, age above 65 should be considered a relative contraindication (2). The most recent guidelines again characterize age over 65 as a relative contraindication but explicitly identify age greater than 75 as the threshold at which individuals are unlikely to be candidates (3). A combination of population-level demographic changes, revision of the lung allocation score (LAS), and clearer data on outcomes in elderly transplant recipients has driven the steady increase in the threshold at which age is taken into consideration and the strength of that recommendation. In this article, we review the current state of lung transplantation in elderly patients.

Factors driving increased transplantation in older patients

In the United States (US), the portion of lung transplant recipients over age 65 has grown 430% over the last 13 years from 6.9% in 2004 to 29.6% in 2016 (*Figure 1*). Internationally, recipients over age 65 increased from 2.6% to almost 17% over a similar time. In both populations, the proportion of transplant recipients over 70 increased from less than half a percent to almost 3% by mid-2012. Demographic, clinical, and lung allocation-related reasons accounted for these changes.

Aging population

With the exception of cystic fibrosis (CF) and certain forms of pulmonary arterial hypertension, the incidence and prevalence of end-stage lung diseases increase with age.
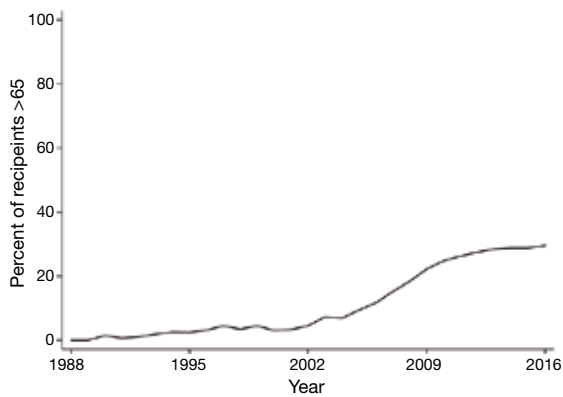
Figure 1 Trends in lung transplantation in the US among recipients >65.
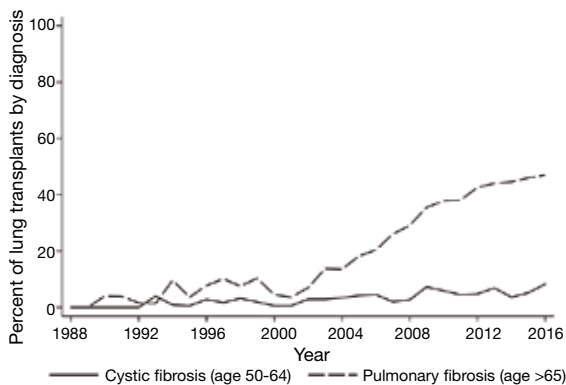

Figure 2 Trends in lung transplantation in the US among older age groups in cystic fibrosis and pulmonary fibrosis.

For example, idiopathic pulmonary fibrosis (IPF), one of the leading indications for transplantation, is significantly more common after age 60 and appears to have the highest prevalence among individuals >70 (4,5). While the epidemiology of IPF varies by geographic region, most studies have reported an increased disease prevalence over the last twenty years, particularly in individuals >65 (6). Concordant with this change, the percentage of IPF transplants performed in patients over age 65 in the US has risen from 7.8% in 1996 to 20.4% in 2006 to 40.7% in 2016 (*Figure 2*). Single center studies have also shown an increase in the percent of patients with IPF referred for transplantation evaluation, from 19.8% in the early 1990s to 30.8% in the mid-2000s (7,8). Larger database studies are necessary to confirm referral trends, although the overall increase in transplants performed for patients with IPF is at least, in part, a function of the rising prevalence of the

disease in an aging population.

Treatment for younger patients

At the same time, advances in the care of patients with genetic lung diseases, particularly CF, has increased survival and delayed the need for lung transplantation, when necessary, until older age (9). Although we are unaware of any published data on the average age of referral for lung transplant in CF, the fraction of lung transplants performed among CF patients above 50 has steadily increased from 2.8% in 1996 to 4.5% in 2006 to 8.3% in 2016 (*Figure 2*). Similarly, the proportion of CF transplants for patients >65, while still extremely small, has steadily increased over the past 20 years. The advent of targeted therapy to improve CFTR function, thereby slowing the decline in lung function, is anticipated to delay the need for transplantation in the CF population, thereby further increasing age at referral (10).

LAS changes

Although the increase in median age of lung transplant recipients began in the late 1990s, the 2005 revision of the LAS accelerated this trend in the US. Prior to that year lungs were allocated based on duration of waitlist time after matching for size and ABO blood type. In May 2005, the LAS was changed to emphasize medical need rather than wait time. The new scoring system weighed anticipated 1-year survival without transplant—so called wait list urgency—twice as heavily as predicted 1-year survival following transplant. Age was included as a variable that impacted the calculation of both waitlist and post-transplant survival although age and LAS score are not related in a linear manner. More importantly than the inclusion of age itself, the LAS gave increased priority to diseases with higher short-term mortality such as IPF that are also more common in older patients. As a consequence, the LAS shifted transplant toward older, sicker recipients (11).

Program willingness

As revisions in allocation schemes and clinical and demographic changes have driven lung transplantation toward older patients, centers have gained greater familiarity with transplantation in this population. In general, however, elderly transplant recipients tend to cluster in high volume programs, suggesting that there is

some combination of willingness and experience with this population that has driven increased transplantation. For example, in the LAS era, recipients >70 are transplanted in centers with an average volume of 34 transplants per year and the increase in lung transplantation in patients >70 appear to have primarily occurred in centers that perform more than 30 transplant per year (12,13). It is unclear to what extent "specialized" experience in the care of elderly recipients has allowed these centers to increase transplants in this population and the extent to which smaller volume centers could expect similar outcomes.

Outcomes in older patients

Perioperative morbidity and mortality

Despite concerns that elderly patients may have reduced functional reserve, placing them for increased risk for perioperative mortality; several large studies have shown no difference in in-hospital or 30-day mortality for older recipients (14). For example, Biswas Roy *et al.* found a 96% survival at 30 days in single lung transplant recipients <65 compared to 96% for those 65–69, 97% for those 70–74, and 96% for those 75–79. Bilateral recipients had similar age-related 30-day survival with the exception of those 75–79 where survival was only 73% (15). An analysis of the US Medicare claims database suggests no difference in in-hospital, 30 or 90-day mortality for recipients >70 compared to those 60–69 and those <60 (13). In this cohort and others, recipients >70 have similar length of hospital stay to those 60–69 and shorter length of stay compared to those <60, possibly reflecting increased use of single lung transplant in this population (13,14). Older recipients, however, have higher rates of 30- and 90-day readmission and age is an independent risk factor for discharge to an inpatient rehabilitation facility following transplant (13,16).

Long-term survival and cause of death

Several studies of long-term survival before the LAS era found reduced long-term survival for older transplant recipients. For example, Gutierrez *et al.* reported a 37% *vs.* 57% adjusted 5-year survival for recipients >60 *vs.* those below 60. Similarly, larger cohort studies of US lung transplants before 2005 demonstrated that age >70 was a significant, independent risk factor for 1- and 3-year mortality (14,17). Studies in the post-LAS era, however, have found conflicting results in survival differences between

patients 60–69 years old and >70 with some reporting equivalent 1-year survival and others showing slightly worse 1- and 3-year survival, depending on the population (13,14). There are consistent data, however, that recipients over 65 have worse mortality than those below 65. For example, the 3-year survival rate for single lung transplant in 12–64 *vs.* 65–69 *vs.* 70–74 year olds drops from 65% to 57% to 49% and from 68% to 63% to 57% for bilateral transplants. Finally, despite case reports of successful transplantation in octogenarians, large cohort studies have consistently shown poor intermediate and long-term outcomes for recipients over age 75 (18). A recent study of survival in recipients over 75 demonstrated 1- and 3-year survival of 51% and 26%, respectively. In the same study, 5-year survival could not be calculated because of the small number of long-term survivors (15).

Despite early concerns that older recipients may be more susceptible to severe infections because of immunosenescence, recent large cohort studies have demonstrated no difference in the incidence of fatal infection in recipients >65 and >70 compared to younger recipients (15,17,18). This may be a function of protocols designed to reduce induction and maintenance immunosuppression in these groups (19). Rates of fatal malignancies and vascular events appear to be more common in patients >65 and >70 (14,16,17). Graft failure as a cause of death, however, declines significantly in patients >70 (14). Single center studies have also suggested that older recipients are at higher risk for drug toxicity, non-fatal malignancies (particularly skin cancer), and cognitive decline suggesting increased need for drug monitoring and dermatologic surveillance in this population (20,21).

Health related quality of life (HRQL)

Given their increased morbidity following transplant, it might be expected that the HRQL benefit would be less for older patients. Cohort studies, however, have conflicted regarding the impact of age on the survival-adjusted HRQL benefit of transplant. Singer *et al.* found, in a single center prospective study, that the significant improvements in multiple HRQL assessments following transplant were not meaningfully different by recipient age, when treated as a continuous variable (22). Only 14% of their cohort (46 patients) were over age 65, however, and only 2% (8 patients) were over age 70 and they did not specifically examine HRQL in these populations compared to younger recipients. In a separate cohort study, researchers found

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that, although patients over 65 did have increases in HRQL scores following transplant, they had substantially smaller changes compared to younger patients (23). Finally, in a large cohort study of US transplants from 2005–2009, there was no difference in rate of functional decline, as measured by Karnofsky performance score, in recipients 65–69 and those >70 compared to younger recipients. Recipients older than 70, however, had worse overall post-transplant functional status than those 65–69, who, in turn, had worse functional status than those <65 (13,24).

Appropriate candidate and procedure selection

In order to maintain equivalent or near-equivalent morbidity and short-term mortality for elderly *vs.* non-elderly recipients, careful candidate, donor, and procedure (single *vs.* bilateral transplant) selection is paramount. Chief among these considerations is differentiating chronologic age from functional status and the accumulation of aging-related comorbidities.

Candidate selection

In the largest cohort studies demonstrating similar perioperative outcomes for older (>65 and >70) recipients, older patients had notably few co-morbidities and were relatively healthy at the time of transplant. For example, Hayanga *et al.* found that almost no recipients above 70 had an abnormal creatinine or total bilirubin; that only 16.8% had diabetes; that only 13.4% were hospitalized at the time of transplant (6.1% in the ICU); and that only 1.2% required intra-operative extracorporeal membrane oxygenation (ECMO) support (14). Similarly, Biswas Roy *et al.* reported that, among transplant recipients >75, only 1% were ventilated pre-transplant and that mean pulmonary arterial pressure was significant lower [22.6 *vs.* 27.5 millimeters mercury (mmHg)] compared to recipients <65 (15). Despite case reports of successful use of ECMO as a bridge to transplantation in patients above 70 years, there are no data to support the routine use of pre-transplant mechanical ventilation or circulatory support in this population (25).

Although there are limited comparative data on frailty measures in elderly *vs.* non-elderly recipients, given the association between frailty and increased post-transplant mortality, it is likely that centers also consider objective and subjective measures of frailty when accepting elderly candidates for listing (26). Similarly, there are no data on transplantation in elderly patients with vascular co-morbidities such as coronary artery disease, carotid disease, or peripheral vascular disease or other age-related diseases such as swallowing dysfunction or neurocognitive impairment. In general, however, ideal elderly candidates should be free from other organ system dysfunction that might negatively impact their post-transplant course.

Donor selection

Given the worse long-term survival among elderly transplant candidates, there has been some consideration of using lungs from extended criteria donors where reduced baseline pulmonary function may be less relevant compared to a younger candidate with longer life expectancy. For example, several single center studies have found that older recipients are more likely to undergo transplantation with lungs from donors above age 55, the typical age cutoff for considering a donor acceptable rather than ideal (20,27,28). In large cohort studies, the use of lungs from donors >55 does not appear to adversely impact overall or conditional one year survival in recipients >65 (29). There are little data, however, on the use of donors with multiple marginal criteria such as older age and significant smoking history. Cohort studies that have demonstrated acceptable outcomes for older recipients have consistently reported a low rate of utilization of lungs from donors with any smoking history and the use of donors with >20 pack-year smoking has been associated with increased mortality in recipients >60 (14,30). Finally, single center data suggest that older recipients are less like to undergo cytomegalovirus (CMV) mismatch (donor positive, recipient negative) transplants, particularly as this has been identified as a significant risk factor for death in the elderly lung transplant population (21).

Procedure selection

The choice of whether to perform single or bilateral transplant in older recipients is an area of ongoing controversy. Although bilateral transplant conveys a significant overall survival benefit for most end-stage lung diseases, association with increased early mortality compared to single lung transplant led to early recommendations to prefer single lung for recipients >65 or even >60 (1,31,32). Advances in surgical technique, ICU and post-operative care, and immunosuppression, however, began to improve survival in elderly recipients and subsequent studies in the post-LAS era have shown increased willingness to perform bilateral transplants in these patients, including those >70

(12,20,33). In a recent study of US transplants in the post-LAS era, Biswas Roy *et al.* found no difference in 30- or 90-day survival in recipients 65–69 or 70–74 with single *vs.* bilateral transplant (15). They did, however, identify a trend to increased overall survival with bilateral transplant in these age groups, independent of underlying obstructive or restrictive lung disease. There was no such benefit apparent in patients above 75. Based on these data, it may be reasonable to consider bilateral transplant in patients <74, assuming no other technical or acuity of illness reasons to prefer single lung transplant. Even with bilateral transplant, however, it is important to note that overall survival remains worse in patients >70 compared to younger recipients (13,15).

Resource allocation considerations

Duty to rescue and fair innings

In weighing predicted mortality without transplant—wait list urgency—more heavily than predicted one year survival following transplant, the revised LAS prioritizes a duty to rescue over maximizing post-transplant survival benefit (34). Coupled with the changing demographics of end-stage lung disease, this has led to increased allocation to older patients, who, at the extreme, derive less long-term survival benefit from transplantation. Surveys of community beliefs about appropriate organ allocation have consistently identified prioritizing younger age—and the chance to live a normal life span or to have a fair number of life "innings"—as important to equitable allocation (35). As with current lung transplantation guidelines, respondents in these community-based surveys do not identify a specific age cut-off, but there is an ongoing tension between a commitment to fair innings and to a system that increases the number of elderly transplant recipients. While this debate continues, an ongoing commitment to careful candidate selection and post-transplant care targeted to the specific needs of this population can help to continue to improve their outcomes.

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Footnote

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References

1. Maurer JR, Frost AE, Estenne M, et al. International guidelines for the selection of lung transplant candidates. The International Society for Heart and Lung Transplantation, the American Thoracic Society, the American Society of Transplant Physicians, the European Respiratory Society. Transplantation 1998;66:951-6.
2. Orens JB, Estenne M, Arcasoy S, et al. International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2006;25:745-55.
3. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015;34:1-15.
4. Nalysnyk L, Cid-Ruzafa J, Rotella P, et al. Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature. Eur Respir Rev 2012;21:355-61.
5. Collard HR, Ward AJ, Lanes S, et al. Burden of illness in idiopathic pulmonary fibrosis. J Med Econ 2012;15:829-35.
6. Raghu G, Chen SY, Yeh WS, et al. Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001-11. Lancet Respir Med 2014;2:566-72.
7. Egan TM, Trulock EP, Boychuk J, et al. Analysis of referrals for lung transplantation. The Washington University Lung Transplantation Group. Chest 1991;99:867-70.
8. de Pablo A, Juarros L, Jodra S, et al. Analysis of patients referred to a lung transplantation unit. Transplant Proc 2013;45:2351-6.
9. MacKenzie T, Gifford AH, Sabadosa KA, et al. Longevity of patients with cystic fibrosis in 2000 to 2010 and beyond: survival analysis of the Cystic Fibrosis Foundation patient registry. Ann Intern Med 2014;161:233-41.
10. Polenakovik HM, Sanville B. The use of ivacaftor in an adult with severe lung disease due to cystic fibrosis (ΔF508/G551D). J Cyst Fibros 2013;12:530-1.
11. Kozower BD, Meyers BF, Smith MA, et al. The impact of the lung allocation score on short-term transplantation outcomes: a multicenter study. J Thorac Cardiovasc Surg 2008;135:166-71.
12. Kilic A, Merlo CA, Conte JV, et al. Lung transplantation in patients 70 years old or older: have outcomes changed

after implementation of the lung allocation score? J Thorac Cardiovasc Surg 2012;144:1133-8.

13. McCarthy F, Savino D, Graves D, et al. Cost and Readmission of Single and Double Lung Transplantation in the U.S. Medicare Population. J Heart Lung Transplant 2017;36:S115.

14. Hayanga AJ, Aboagye JK, Hayanga HE, et al. Contemporary analysis of early outcomes after lung transplantation in the elderly using a national registry. J Heart Lung Transplant 2015;34:182-8.

15. Biswas Roy S, Alarcon D, Walia R, et al. Is There an Age Limit to Lung Transplantation? Ann Thorac Surg 2015;100:443-51.

16. Tang M, Mawji N, Chung S, et al. Factors affecting discharge destination following lung transplantation. Clin Transplant 2015;29:581-7.

17. Gutierrez C, Al-Faifi S, Chaparro C, et al. The effect of recipient's age on lung transplant outcome. Am J Transplant 2007;7:1271-7.

18. Shigemura N, Brann S, Wasson S, et al. Successful lung transplantation in an octogenarian. J Thorac Cardiovasc Surg 2010;139:e47-8.

19. Mahidhara R, Bastani S, Ross DJ, et al. Lung transplantation in older patients? J Thorac Cardiovasc Surg 2008;135:412-20.

20. Vadnerkar A, Toyoda Y, Crespo M, et al. Age-specific complications among lung transplant recipients 60 years and older. J Heart Lung Transplant 2011;30:273-81.

21. Cohen DG, Christie JD, Anderson BJ, et al. Cognitive function, mental health, and health-related quality of life after lung transplantation. Ann Am Thorac Soc 2014;11:522-30.

22. Singer LG, Chowdhury NA, Faughnan ME, et al. Effects of Recipient Age and Diagnosis on Health-related Quality-of-Life Benefit of Lung Transplantation. Am J Respir Crit Care Med 2015;192:965-73.

23. Singer JP, Katz PP, Soong A, et al. Effect of Lung Transplantation on Health-Related Quality of Life in the Era of the Lung Allocation Score: A U.S. Prospective Cohort Study. Am J Transplant 2017;17:1334-45.

24. Genao L, Whitson HE, Zaas D, et al. Functional status after lung transplantation in older adults in the post-allocation score era. Am J Transplant 2013;13:157-66.

25. Hayanga JA, Murphy E, Girgis RE, et al. Extracorporeal Membrane Oxygenation as a Bridge to Lung Transplantation in Patients Over Age 70 Years: A Case Report. Transplant Proc 2017;49:218-20.

26. Wilson ME, Vakil AP, Kandel P, et al. Pretransplant frailty is associated with decreased survival after lung transplantation. J Heart Lung Transplant 2016;35:173-8

27. Sommer W, Ius F, Salman J, et al. Survival and spirometry outcomes after lung transplantation from donors aged 70 years and older. J Heart Lung Transplant 2015;34:1325-33.

28. Shigemura N, Horai T, Bhama JK, et al. Lung transplantation with lungs from older donors: recipient and surgical factors affect outcomes. Transplantation 2014;98:903-8.

29. Hayes D Jr, Black SM, Tobias JD, et al. Influence of donor and recipient age in lung transplantation. J Heart Lung Transplant 2015;34:43-9.

30. Nwakanma LU, Simpkins CE, Williams JA, et al. Impact of bilateral versus single lung transplantation on survival in recipients 60 years of age and older: analysis of United Network for Organ Sharing database. J Thorac Cardiovasc Surg 2007;133:541-7.

31. Fischer S, Meyer K, Tessmann R, et al. Outcome following single vs bilateral lung transplantation in recipients 60 years of age and older. Transplant Proc 2005;37:1369-70.

32. Meyer DM, Bennett LE, Novick RJ, et al. Single vs bilateral, sequential lung transplantation for end-stage emphysema: influence of recipient age on survival and secondary end-points. J Heart Lung Transplant 2001;20:935-41.

33. Palmer SM, Davis RD, Simsir SA, et al. Successful bilateral lung transplant outcomes in recipients 61 years of age and older. Transplantation 2006;81:862-5.

34. McShane PJ, Garrity ER Jr. Impact of the lung allocation score. Semin Respir Crit Care Med 2013;34:275-80.

35. Tong A, Howard K, Jan S, et al. Community preferences for the allocation of solid organs for transplantation: a systematic review. Transplantation 2010;89:796-805.

Outcomes after lung transplantation

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Abstract: With more than 50,000 procedures having been performed worldwide, lung transplantation (LT) has become the standard of care for patients with end-stage chronic respiratory failure. LT leads to dramatic improvements in both pulmonary function and health related quality of life. Survival after LTs has steadily improved, but still lags far behind that observed after other solid organ transplantations, as evidenced by a median survival rate that currently stands at 5.8 years. Because of these disappointing results, the ability of LT to expand survival has been questioned. However, the most recent studies, based on sophisticated statistical modeling suggest that LT confers a survival benefit to the vast majority of lung transplant recipients. Chronic lung allograft dysfunction (CLAD) that develops in about 50% of recipients 5 years after LT is a major impediment to lung transplant survival. A better understanding of the mechanisms underlying CLAD could allow for better post-transplant survival.

Keywords: Lung transplantation (LT); outcome; survival; quality of life

Introduction

Although lung transplantation (LT) has become an invaluable approach for the treatment of end-stage respiratory disease, survival after the procedure is not yet as good as that after other solid-organ transplants (1). Because of this, patient survival has been the primary outcome measurement in most studies. Other indicators of outcomes like pulmonary function or quality of life have also been studied.

Survival

To date, the registry of the International Society for Heart and Lung Transplantation (ISHLT) has accrued data on more than 55,000 adult patients who received a LT in about 250 lung transplant centers from the early 90s (2). This registry provides invaluable information regarding lung transplant activity and outcome.

According to the 2016 report of this registry, adult patients who underwent primary LT between January 1990 and June 2014 had a median survival of 5.8 years, with unadjusted survival rates of 89% at 3 months, 80% at 1-year, 65% at 3 years, 54% at 5 years and 32% at 10 years (2). Post-transplant survival has improved over time with a median survival of 4.2 years in the 1990–1998 era compared to 6.1 years in the 1999–2008 era. It is remarkable that post-transplant survival continued to increase in spite of considerable change in patients' characteristics and severity at the time of transplant. In the US for instance, between 2002 and 2014, the proportion of patients aged more than 65 years old rose from 4.5% to 28.7%, the proportion of patients being in the ICU rose from 4.2% to 15.5%, the proportion of patients under mechanical ventilation doubled, and the proportion of patients under ECMO reached 2.2% (3,4). The same shift in patient case-mix has been observed in European countries with the development of organ allocation in high emergency (5,6). Despite

these improvements, survival outcomes for LT recipients remain inferior to those achieved after other solid-organ transplant procedures. For instance, the median survival after heart transplantation in the same registry is around 12 years (2).

A closer look at the LT survival curves shows that there is a large drop in early survival in the first months following LT followed by a slow attrition over time. Improvements in the management of patients in the early post-operative period led to a reduction in early mortality over the years. To this regard, some centers report on 1-year mortality well below 10% (7). However, the attrition rate after the first year, which is mainly attributable to chronic lung allograft dysfunction (CLAD) that develops in 50% of grafts at 5 years, remains largely unchanged (2).

One of the main determinants of LT outcome is the underlying disease, with a median survival of 8.9 years for cystic fibrosis (CF) patients, 6.7 years for chronic obstructive pulmonary disease (COPD) with alpha-1 antitrypsin deficiency (AATD), 5.6 years for COPD without AATD, 4.8 years for idiopathic interstitial pneumonia and 2.8 years for re-transplantation. These differences seem to be more related to differences in patients' characteristics at the time of LT than to the underlying disease by itself. For instance, patients with COPD are older, more frequently tobacco smokers and have more comorbidities than patients with CF.

Other prognostic factors are related either to the recipient (gender, age, 6 min walking distance, patient under mechanical ventilation, dialysis or hospitalized in ICU), the donor (diabetes, age, gas exchange at the time of harvest, cause of death), the donor/recipient interaction (number of HLA mismatches, CMV or gender mismatch), the surgical approach (single *vs.* bilateral) and the center volume (2,8). The role of other factors like size mismatch or graft ischemic time is more debated (9-11).

Although most of these factors are not alterable, the surgical approach is. The choice between single and bilateral LT has been debated for a long time. Although the vast majority of patients with suppurative lung diseases (including CF) receive a bilateral LT (BLT), the choice of procedure remains a matter a debate for patients with COPD and IPF. Unadjusted survival rates are in favor of BLT with a median survival of 7.3 years compared to 4.6 years for single LT (SLT) recipients according to the ISHLT registry (12). However, SLT is in general proposed to older and more frail patients and analyses adjusted for patients characteristics showed conflicting results (13-15).

In the absence of randomized controlled trial it is difficult to draw definitive conclusions, and the evidence comes mainly from the analysis of large registries. In COPD patients, Thabut *et al.* found a better survival after BLT especially in patients aged more than 60 years old (14). Schaffer *et al.*, using more recent data but closely related methods, did not find a statistically significant difference between both surgical approaches in this indication (15). In IPF patients, although Thabut *et al.* failed to detect any difference in survival between both procedures (13), Schaffer *et al.* found better adjusted survival after BLT (15). These differences may be explained in part by differences in the characteristics of patients between the two studies, the study by Schaffer *et al.* including patients receiving a LT after LAS implementation.

It must be kept in mind that most of the evidence about post-transplant survival comes from large registries that lump together the outcomes of transplantations performed many years ago in centers that no longer exist with those performed in the recent years in high volume centers.

Survival benefit

Given the disappointing long-term survival of patients after LT, its ability to extend survival has been questioned (16,17). In the absence of randomized trials, appraisal of the survival benefit of LT is complex and relies on statistical modeling (18). These approaches have to deal with the following issues: patients referred to a LT center form a very selected subgroup of patients with the disease of interest, and patients who ultimately receive a LT form a selected subgroup of patients who are put on a waiting list, that may not be reflected by the characteristics of patients measured at the time of registration (18). Methods taking into account the evolution of patients' characteristics after registration have recently been developed and provide more sensible estimates of the survival benefit of LT (18,19). Besides these technical issues, the reader must keep in mind that the results of these studies are valid for a given organ allocation system and may not apply for transplantations performed in the same indication, but in another country and may not be valid 10 years from now, because of the evolution of both pre- and post-transplant survival. In the case of CF for instance, the spontaneous life expectancy improved from 31 to 37 years over the past decade and new drugs able to dramatically change the expected survival have been developed recently (20).

About 20 studies have been published that aimed to

assess the survival benefit of LT in different indications (14,16,17,19,21-34). These studies are summarized in *Table 1*. The survival benefit of LT is best documented in patients with IPF and CF whereas it is still debated in COPD and a lack of data precludes definitive conclusion in pulmonary arterial hypertension (PAH). In the case of COPD, a few prognostic factors have shown association with post-transplant outcome and could be used as markers to refine patient's selection.

Quality of life

One of the main clinical aims of LT is to improve quality of life, and may be the only expected clinical benefit of LT in some indications like COPD where the survival benefit is still unclear. Quality of life encompasses many subdomains like financial status, social support, physical environment and health (36). Many studies have been published in the recent years that focused on health-related quality of life (HRQoL) before and after LT. However, the interpretation of these studies is not trivial. First, there are plenty of instruments available to measure HRQoL. Some of these instruments are generic (36-item Short Form Survey—SF-36 for instance) whereas others are disease-specific (SGRQ). In some cases, health utility measures are used that can be combined with survival to derive quality adjusted life survival. In a recent systematic review focusing on the estimation of HRQoL after LT, Seiler *et al.* retrieved 39 studies that used 13 different HRQoL instruments (37). Although all these instruments have advantages and limitations, they do not explore the same domains and are thus likely not to provide the same estimation of the benefit of LT. Second, a major limitation of these studies is related to the fact that patients must be well enough to fill out the questionnaires. In other words, in these studies, the data are not missing at random. For instance, in a study published 10 years ago, the authors report on the HRQoL of patients before and after LT using the SGRQ (38). In this study, patients who died after LT were excluded. This study does not allow to conclude on the improvement in HRQoL provided by LT, but only on the improvement of HRQoL in patients doing well after LT. Several methods have been used to account for these missing not at random data, like imputing the worst possible HRQoL to those who died post-transplant or combining survival and HRQoL (39).

All the studies focusing on HRQoL after LT found dramatic improvements in HRQoL regardless of the indication for LT and whether HRQL is measured by generic, respiratory-specific HRQL instruments, or by utility measures (36,37). *Table 2* reports the benefit of LT on quality of life measured by both generic and specific tools.

The most popular generic HRQoL instrument is the SF-36. The SF-36 features physical and mental summary scores (PCS and MCS), and a 4-point change in the SF-36 is considered clinically significant (MCID). In a multicenter randomized controlled trial about CMV prophylaxis, SF-36 was measured before transplantation and every 3 months up to 1 year after LT (41). The authors observed a 10.9 points improvement in PCS score, almost reaching the norms of the US population. Concomitant with increased PCS scores, they also found increase in the subdomains that contribute to PCS: physical function, role-physical, and general health. In contrast, the MCS did not change from baseline level, remaining well below the US population norm throughout the first post-operative year. Further evaluation of the MCS domains showed that mental health and vitality domain scores did not improve, whereas increases were observed in social function and role-emotional domains. In a recent prospective study involving 326 patients that contributed to HRQoL measurements both before and after LT, a 17.7 improvement in the SF-36 physical component score was observed (39). Again, the improvement in the mental component score was more modest (7.8 points). Other studies using the SF-36 or other HRQoL instruments have reported mostly the same results. Similar results were found in studies performed after the introduction of the LAS score in the US (40).

The same results have been found in studies using respiratory-specific HRQoL. One of the most popular disease-specific HRQoL tool is the St. George's Respiratory Questionnaire (SGRQ) that provides a summary score and a score for 3 sub-domains: impact, symptoms and activity. In a prospective cohort study involving 326 patients in whom HRQoL has been measured pre and post transplantation using various questionnaires, average improvements in SGRQ was 47 points, which is more than 10 times the MCID for this tool (39). These changes greatly exceed those seen with other treatments for advances lung disease. For instance, in recent studies on bronchoscopic lung volume reduction (BLVR) in COPD patients, the mean improvements in total SGRQ was 13.4 points (42) to be compared to 49.9 points improvements in patients receiving a LT in the study by Singer *et al.* (39).

Table 1 Studies assessing the survival benefit of lung transplantation

First author (reference)	Publication year	Diseases	Study type	Cohort period	Main conclusion
Hosenpud (17)	1998	Adult CF, COPD, ILD	UNOS Registry, US	1992–1994	LT improves survival in patients with CF and ILD, no benefit for patients with COPD
Geertsma (31)	1998	Adult CF, COPD, ILD, PAH	Single center, Netherlands	1990–1996	LT improves survival for the whole cohort of patients. Disease specific analysis limited by the small sample size
Aurora (34)	1999	Pediatric CF	Single center, UK	1988–1998	LT improves survival in children with CF
Liou (28)	2001	Pediatric and adult CF	UNOS registry, US	1992–1997	LT improves survival for patients with CF and a predicted 5-year survival <5 years. Most patients with CF have unclear or even negative survival effect
De Meester (32)	2001	Adult CF, COPD, ILD, PAH	Eurotransplant registry	1990–1996	LT improves survival in all indications except Eisenmenger syndrome
Charman (33)	2002	Adult CF, COPD, ILD, PAH	Single center, UK	1984–1999	LT improves survival in all indications except Eisenmenger syndrome
Thabut (22)	2003	ILD	Single center, France	1988–2001	LT improves survival for patients with ILD
Liou (27)	2005	Pediatric and adult CF	UNOS registry, US	1988–2002	LT improves survival in adult patients with CF, a 5-year predicted survival <50% and no Burkholderia cepacia or arthropathy. No benefit in pediatric CF
Stavem (25)	2006	COPD	Single center, Norway	1990–2003	LT does not improve survival in patients with COPD
Liou (16)	2007	Pediatric CF	UNOS registry, US	1998–2004	LT improves survival for <1% of pediatric CF patients
Thabut (35)	2008	COPD	UNOS registry, US	1987–2004	LT improves survival by at least 1-year for 45% of COPD patients undergoing BLT and for 22% undergoing SLT
Titman (21)	2009	Adult CF, COPD, ILD, PAH	National registry, UK	1995–2006	LT improves survival in all patients
Hofer (30)	2009	Pediatric and adult CF	Single center, Switzerland	1992–2007	LT improves survival in children and adult patients with CF
Lahzami (29)	2010	COPD	2 centers, Switzerland	1993–2007	LT improves survival for patients with COPD and a BODE >7
Tanash (24)	2011	AATD related emphysema	National registry, Sweden	1990–2010	LT improves survival in AATD patients with emphysema
Russo (26)	2011	All patients >12 years old	UNOS, US	2005–2009	LT improves survival in patients with a LAS >40
Thabut (23)	2013	Adult CF	UNOS, US	2005–2009	LT improves survival in adult CF patients
Vock (19)	2017	Adult CF, COPD, ILD, PAH	UNOS, US	2005–2011	Almost ¾ of patients achieve a 2-year survival benefit

CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; AATD, alpha-1 antitrypsin deficiency; LT, lung transplantation; BLT, bilateral lung transplantation; SLT, single lung transplantation.

Table 2 Benefit of lung transplantation on quality of life measured by both generic and specific tools, according to the underlying disease (these measures are made during the first year following lung transplantation)

Type	Tool	Underlying disease	Value, mean (range)	First author (reference)
Generic	SF12-PCS (MCID =5)	COPD	15.9 (11.5–20.3)	Singer (40)
		PAH	7.9 (1.0–14.7)	
		CF	23.8 (19.5–28.1)	
		IPF	13.8 (11.9–15.8)	
	SF12-MCS (MCID =5)	COPD	2.7 (−0.9–6.4)	
		PAH	0.1 (−5.5–5.7)	
		CF	10.3 (6.4–14.1)	
		IPF	4.8 (3.1–6.6)	
	EQ5D (MCID =0.06)	COPD	0.15 (0.08–0.21)	
		PAH	0.07 (−0.05–0.19)	
		CF	0.30 (0.22–0.39)	
		IPF	0.16 (0.13–0.19)	
	SF-36 PCS (MCID =4)	COPD	18.3 (16.4–20.1)	
		PAH	18.0 (14.6–21.3)	
		CF	19.6 (17.5–21.8)	
		IPF	15.4 (13.6–17.1)	
	SF-36 MCS (MCID =4)	COPD	8.4 (6.4–10.4)	
		PAH	7.7 (4.0–11.3)	
		CF	9.1 (6.7–11.5)	
		IPF	4.4 (2.5–6.3)	
Specific	SGRQ (MCID =4)	COPD	47.7 (44.3–51.0)	Singer (39)
		PAH	36.3 (30.3–42.3)	
		CF	46.0 (42.0–49.9)	
		IPF	38.5 (35.4–41.7)	
Utility	QALYs	COPD	2.33 (2.03–2.63)	Singer (39)
		PAH	2.53 (2.02–3.04)	
		CF	2.87 (2.53–3.20)	
		IPF	2.17 (1.90–2.44)	

This table does not intend to summarize all the data available, but is a selection of a few recent studies reporting quality of life benefit according to the underlying disease. QALYs, quality adjusted life years; SGRQ, St. George's Respiratory Questionnaire.

In another study, an improvement of 33 points was found, with similar improvements in the 3 domain scores (43). This improvement persisted even when the worst possible values were imputed to patients who died after LT. These improvements were similar to those found after other solid organ transplantations (44).

Most studies focused on the first years following LT and very few studies reported on QoL of patients surviving more than 3 years after LT. As such, the trajectory of QoL beyond 3 years post-transplant remains uncertain. A few

factors have been associated with post-transplant QoL. CLAD developing in about 50% of patients at 5 years appears to be the strongest determinant of physical health status (45). The other predictors of HRQoL after LT were immunosuppressants side-effects, indication for LT, older age at the time of transplant, a single-lung transplant, and recurrent infections (37).

Pulmonary function tests

The pulmonary function of transplant recipients results from pre-transplant factors (underlying disease in the case of SLT), operative factors (pleural or diaphragmatic injury) and post-transplant complications (bronchial strictures). In the first weeks after LT, pulmonary function is hampered by various factors including pain and early graft dysfunction, and the peak in pulmonary function is in general observed between 3 to 12 months following LT. The average function declines thereafter because of CLAD that develops in 50% of patients at 5 years. Surgical approach (SLT *vs.* BLT) and underlying disease in case of SLT are the two main factors associated with post-transplant pulmonary function.

Patients who receive a BLT typically achieve normal pulmonary function tests (FEV_1, FVC, TLC) as well a gas exchange whatever the indication for LT (46). Lower PFTs are achieved following SLT and depend on the indication. Almost normal FEV_1 can be expected in patients with PAH, whereas IPF patients have typically FEV_1 between 60 and 80 percent of predicted value and COPD patients achieve typically FEV_1 in the 50–60% range (47). Blood gases are typically normal. Small sample size studies performed many years ago have shown that considerable exercise limitations persisted after either single or bilateral LT despite pulmonary function restoration, with VO_2 around 50% of predicted values (48). Similar results were found in a study including 153 patients in recent years (49,50). Interestingly, BLT did not result in better exercise tolerance than SLT (49,50). The skeletal muscle appears to be the cause of exercise limitation in most and may, in part, reflect persistence of a pre-transplant skeletal muscle injury (46).

Other outcomes have been reported like employment status. For instance, in the ISHLT registry, at 5 years post-transplant, about 40% of patients are not working, 30% are retired and a little less than 20% are working part or full-time. However, these figures are likely to vary from country to country and pose the same issues of missing values at that already mentioned for HRQoL.

In conclusion, LT allows for major improvements in lung function and exercise tolerance that translates into dramatic improvement in HRQoL that far exceeds the effects of other treatments of end-stage lung diseases. Although recent studies suggest that LT improves survival in most cases, post-transplantation survival remains hampered by the frequent development of CLAD. A better understanding of the mechanisms implicated in CLAD development could allow to match the outcomes after other solid organ transplantations.

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Footnote

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References

1. Kotloff RM, Thabut G. Lung transplantation. Am J Respir Crit Care Med 2011;184:159-71.
2. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant 2016;35:1170-84.
3. Valapour M, Skeans MA, Smith JM, et al. Lung. Am J Transplant 2016;16 Suppl 2:141-68.
4. Valapour M, Skeans MA, Heubner BM, et al. OPTN/ SRTR 2012 Annual Data Report: lung. Am J Transplant 2014;14 Suppl 1:139-65.
5. Lafarge M, Mordant P, Thabut G, et al. Experience of extracorporeal membrane oxygenation as a bridge to lung transplantation in France. J Heart Lung Transplant 2013;32:905-13.
6. Boussaud V, Mal H, Trinquart L, et al. One-year experience with high-emergency lung transplantation in France. Transplantation 2012;93:1058-63.
7. Transplant Center Search Results. Accessed on June 1, 2017. Available online: https://www.srtr.org/transplant-cen ters/?&organ=lung&recipientType=adult&sort=volume
8. Thabut G, Christie JD, Kremers WK, et al. Survival differences following lung transplantation among US transplant centers. Jama 2010;304:53-60.

9. Thabut G, Mal H, Cerrina J, et al. Graft ischemic time and outcome of lung transplantation: a multicenter analysis. Am J Respir Crit Care Med 2005;171:786-91.

10. Eberlein M, Reed RM, Maidaa M, et al. Donor-recipient size matching and survival after lung transplantation. A cohort study. Ann Am Thorac Soc 2013;10:418-25.

11. Eberlein M, Reed RM, Bolukbas S, et al. Lung size mismatch and primary graft dysfunction after bilateral lung transplantation. J Heart Lung Transplant 2015;34:233-40.

12. Registry I. 2016 Slides. Accessed 06/03/2017. Available online: https://www.ishlt.org/registries/slides. asp?slides=heartLungRegistry

13. Thabut G, Christie JD, Ravaud P, et al. Survival after bilateral versus single-lung transplantation for idiopathic pulmonary fibrosis. Ann Intern Med 2009;151:767-74.

14. Thabut G, Christie JD, Ravaud P, et al. Survival after bilateral versus single lung transplantation for patients with chronic obstructive pulmonary disease: a retrospective analysis of registry data. Lancet 2008;371:744-51.

15. Schaffer JM, Singh SK, Reitz BA, et al. Single- vs double-lung transplantation in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis since the implementation of lung allocation based on medical need. JAMA 2015;313:936-48.

16. Liou TG, Adler FR, Cox DR, et al. Lung transplantation and survival in children with cystic fibrosis. N Engl J Med 2007;357:2143-52.

17. Hosenpud JD, Bennett LE, Keck BM, et al. Effect of diagnosis on survival benefit of lung transplantation for end-stage lung disease. Lancet 1998;351:24-7.

18. Thabut G. Estimating the Survival Benefit of Lung Transplantation: Considering the Disease Course during the Wait. Ann Am Thorac Soc 2017;14:163-4.

19. Vock DM, Durheim MT, Tsuang WM, et al. Survival Benefit of Lung Transplantation in the Modern Era of Lung Allocation. Ann Am Thorac Soc 2017;14:172-81.

20. Accurso FJ, Rowe SM, Clancy JP, et al. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. N Engl J Med 2010;363:1991-2003.

21. Titman A, Rogers CA, Bonser RS, et al. Disease-specific survival benefit of lung transplantation in adults: a national cohort study. Am J Transplant 2009;9:1640-9.

22. Thabut G, Mal H, Castier Y, et al. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. J Thorac Cardiovasc Surg 2003;126:469-75.

23. Thabut G, Christie JD, Mal H, et al. Survival benefit of lung transplant for cystic fibrosis since lung allocation score implementation. Am J Respir Crit Care Med 2013;187:1335-40.

24. Tanash HA, Riise GC, Hansson L, et al. Survival benefit of lung transplantation in individuals with severe alpha1-antitrypsin deficiency (PiZZ) and emphysema. J Heart Lung Transplant 2011;30:1342-7.

25. Stavem K, Bjortuft O, Borgan O, et al. Lung transplantation in patients with chronic obstructive pulmonary disease in a national cohort is without obvious survival benefit. J Heart Lung Transplant 2006;25:75-84.

26. Russo MJ, Worku B, Iribarne A, et al. Does lung allocation score maximize survival benefit from lung transplantation? J Thorac Cardiovasc Surg 2011;141:1270-7.

27. Liou TG, Adler FR, Huang D. Use of lung transplantation survival models to refine patient selection in cystic fibrosis. Am J Respir Crit Care Med 2005;171:1053-9.

28. Liou TG, Adler FR, Cahill BC, et al. Survival effect of lung transplantation among patients with cystic fibrosis. Jama 2001;286:2683-9.

29. Lahzami S, Bridevaux PO, Soccal PM, et al. Survival impact of lung transplantation for COPD. Eur Respir J 2010;36:74-80.

30. Hofer M, Benden C, Inci I, et al. True survival benefit of lung transplantation for cystic fibrosis patients: the Zurich experience. J Heart Lung Transplant 2009;28:334-9.

31. Geertsma A, Ten Vergert EM, Bonsel GJ, et al. Does lung transplantation prolong life? A comparison of survival with and without transplantation. J Heart Lung Transplant 1998;17:511-6.

32. De Meester J, Smits JM, Persijn GG, et al. Listing for lung transplantation: life expectancy and transplant effect, stratified by type of end-stage lung disease, the Eurotransplant experience. J Heart Lung Transplant 2001;20:518-24.

33. Charman SC, Sharples LD, McNeil KD, et al. Assessment of survival benefit after lung transplantation by patient diagnosis. J Heart Lung Transplant 2002;21:226-32.

34. Aurora P, Whitehead B, Wade A, et al. Lung transplantation and life extension in children with cystic fibrosis. Lancet 1999;354:1591-3.

35. Thabut G, Ravaud P, Christie JD, et al. Determinants of the survival benefit of lung transplantation in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008;177:1156-63.

36. Singer JP, Singer LG. Quality of life in lung transplantation. Semin Respir Crit Care Med 2013;34:421-30.

37. Seiler A, Klaghofer R, Ture M, et al. A systematic review of health-related quality of life and psychological outcomes after lung transplantation. J Heart Lung Transplant

2016;35:195-202.

38. Gerbase MW, Spiliopoulos A, Rochat T, et al. Health-related quality of life following single or bilateral lung transplantation: a 7-year comparison to functional outcome. Chest 2005;128:1371-8.

39. Singer LG, Chowdhury NA, Faughnan ME, et al. Effects of Recipient Age and Diagnosis on Health-related Quality-of-Life Benefit of Lung Transplantation. Am J Respir Crit Care Med 2015;192:965-73.

40. Singer JP, Katz PP, Soong A, et al. Effect of Lung Transplantation on Health-Related Quality of Life in the Era of the Lung Allocation Score: A U.S. Prospective Cohort Study. Am J Transplant 2017;17:1334-45.

41. Finlen Copeland CA, Vock DM, Pieper K, et al. Impact of lung transplantation on recipient quality of life: a serial, prospective, multicenter analysis through the first posttransplant year. Chest 2013;143:744-50.

42. Deslée G, Mal H, Dutau H, et al. Lung Volume Reduction Coil Treatment vs Usual Care in Patients With Severe Emphysema: The REVOLENS Randomized Clinical Trial. Jama 2016;315:175-84.

43. Eskander A, Waddell TK, Faughnan ME, et al. BODE index and quality of life in advanced chronic obstructive pulmonary disease before and after lung transplantation. J Heart Lung Transplant 2011;30:1334-41.

44. Kugler C, Gottlieb J, Warnecke G, et al. Health-related quality of life after solid organ transplantation: a prospective, multiorgan cohort study. Transplantation 2013;96:316-23.

45. Künsebeck HW, Kugler C, Fischer S, et al. Quality of life and bronchiolitis obliterans syndrome in patients after lung transplantation. Prog Transplant 2007;17:136-41.

46. Williams TJ, Grossman RF, Maurer JR. Long-term functional follow-up of lung transplant recipients. Clin Chest Med 1990;11:347-58.

47. Mal H, Sleiman C, Jebrak G, et al. Functional results of single-lung transplantation for chronic obstructive lung disease. Am J Respir Crit Care Med 1994;149:1476-81.

48. Williams TJ, Patterson GA, McClean PA, et al. Maximal exercise testing in single and double lung transplant recipients. Am Rev Respir Dis 1992;145:101-5.

49. Orens JB, Becker FS, Lynch JP 3rd, et al. Cardiopulmonary exercise testing following allogeneic lung transplantation for different underlying disease states. Chest 1995;107:144-9.

50. Bartels MN, Armstrong HF, Gerardo RE, et al. Evaluation of pulmonary function and exercise performance by cardiopulmonary exercise testing before and after lung transplantation. Chest 2011;140:1604-11.