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ADVANCES IN LUNG TRANSPLANTATION

Editors: Heng Zhao
Daniel Kreisel
Marcelo Cypel

AME Medical Book 1A024

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Room C 16F, Kings Wing Plaza 1, NO. 3 on Kwan Street, Shatin, NT, Hong Kong
Information on this title: www.amegroups.com
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First published in 2018
Printed in China by AME Publishing Company

Editors: Heng Zhao, Daniel Kreisel, Marcelo Cypel
Cover Image Illustrator: Anthony P. Yim, HongKong, China

Advances in Lung Transplantation

(Hard Cover)

ISBN: 978-988-78920-8-3
AME Publishing Company, Hong Kong

Advances in Lung Transplantation
(FIRST EDITION)

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

Finishing my medical degree after 4 years and 3 months of study, I decided to quit going on to become 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 introduced by the narrator 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 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 BMJ and a famous BBC host. Surprisingly, during the award presentation, the speeches made by BMJ never mentioned any high impact papers the teams had published in whichever 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. Whilst having a clear focus on research, we have been adhering to the core value "Patients come first". On April 24, 2014, we developed a public account on WeChat (a popular Chinese social media) 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 rarely 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

Since the first successful lung transplant in 1983 by Joel Cooper and colleagues slow but significant advances have been achieved in this field. So far, approximatively 45,000 recipients have benefitted from this procedure worldwide, and about 4,000 new lung transplants are being performed annually, with an increasing number of centres involved around the world. Increased technical expertise and advances in perioperative care and immunosuppression therapy have led to improved short and long-term survival. Despite significant progresses and successes observed in the last 35 years, several limitations still persist and some challenging problems remain unsolved. Paucity of available donors with still significant mortality for patients in the waiting list, poorer outcome if compared with other solid organs transplant, and still consistent perioperative morbidity represent main critical issues in this setting, which physicians operating in this field have to deal with.

However, lung transplant is currently the only viable treatment for many patients with end stage pulmonary disease not responding to medical or surgical therapies, and patients continue to become sicker and more complex in their comorbidities.

The present volume represents an outstanding overview on main topics in the field of lung transplantation with contributions by authors from some of main centres in the world, which have contributed to advances achieved over last decades.

Immunosuppressive regimens have significantly contributed to the improvement of survival after lung transplantation. Data concerning the use of these agents have been effectively summarized by the Columbia University group pointing out the need for randomized clinical trials to allow the development of better regimens and reduce morbidity and mortality for recipients.

Scientific research in the murine model has been largely developed in recent years and provides great opportunities to understand mechanisms that affect lung allograft survival and explore new therapies. Scientific uses, technical tricks and advances of orthotopic lung transplantation and of retransplantation in the mouse have been described in this volume by the scientists of the Washington University in Saint Louis, based on their large experience in this field.

The criteria defining adequate clinical and physiologic parameters for lung donation have remained substantially unchanged since their determination in the 1980s. These criteria have been herein examined by individual factors evaluating their effects on outcomes. Such analysis, performed by the colleagues of the Universities of Louisville and of Philadelphia, could be of practical utility, especially because, at present many centres advocate the use of extended criteria donors with the aim of increasing the donor pool with similar transplant outcome.

A comprehensive overview of indications and outcomes for both adult and pediatric lung transplantation has been provided in this book by the group of the Ohio State University.

Discussion of technical aspects, with special interest for bilateral sequential lung transplantation and airway anastomosis including management of related complications, have been assigned to two of the groups with the largest experience in the field, those of the Pittsburgh University and of the University of Rome, respectively.

Historically, the use of extracorporeal circulatory support such as ECMO has been found related to poor outcome and therefore considered contraindicated in lung transplantation. However, more recently, many centres are trying to optimize their ECMO strategies as a means of bridging acute high-risk patients for lung transplantation. This topic has been thoroughly exposed by the colleagues of the Duke University who have also provided, in this book, an interesting up-to-date review of the practice and management strategies for lung transplantation based on their experience of over 1600 procedures.

The paucity of suitable donor lungs compared with the increasing number of patients who are candidates for lung transplant reflects in considerable waitlist mortality. Ex vivo lung perfusion has emerged as a new preservation technique whose application in high-risk donor lungs has proved successful in expanding the donor pool. An interesting review of technical details and of results of worldwide clinical experience with this technique is reported in this book by the colleagues of the University of Toronto.

Although lung transplantation is still strongly limited by insufficient donor organ availability, operative or bronchoscopic treatment options including Lung Volume Reduction Surgery, endobronchial valves, vapour or coils are offered only to a minority of patients with advanced Chronic Obstructive Pulmonary Disease (COPD). These techniques have appeared promising in early clinical trial, but further data are needed to better define their role in advanced COPD. The “state of the art” of these procedures has been exposed by the colleagues from the University of Queensland.

I am convinced that all the above mentioned contributions by outstanding authors will interest the readers and will provide a complete overview of a complex topic such as lung transplantation.

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Lung transplantation has come a long way since the first attempt of transplant surgery in humans performed by Dr. James Hardy and his team in 1963 and the world's first successful long-term single lung transplantation performed by Dr. Joel Cooper in 1983 (1). Over the past three decades, immunosuppression, lung donor selection criteria and surgical techniques have improved greatly. In this new book, *Advances in Lung Transplantation*, we aim to provide an overview of recent advances in lung transplantation.

The first article on lung transplantation at Duke provided Duke University Medical Center's rich experience on management strategies for lung transplantation. Transplant surgeons at Duke University Medical Center are experienced in single and double lung transplants, as well as multi-organ transplants and more than 1,600 lung transplants have been performed at Duke since 1992 (2). Through this paper, we could learn more novel knowledge from other center besides our accepted ones.

Immunosuppressive regimens have been playing an important role in reducing acute and chronic rejection after lung transplantation, however, though improvements have been made in immunosuppressive therapy, the long-term outcomes are not satisfactory enough (3). Better agents and regimens are needed to solve the above issue for lung transplant recipients (4).

It is well known that conventional criteria for lung donation was very strict including requirements like clear chest radiographic findings, without lung or cardiac disease and age less than 40, and so on. In order to increase the availability of donor organs, extended criteria has been employed by many centers. In the donor selection criteria article, individual factors that may impact outcomes of lung transplantation were examined and it tried to improve our understanding of the context of ideal criteria (5).

With the rapid development of surgical techniques, we are pleased to see that the incision is getting smaller and the hospital stay is dramatically shortened. Though great progress has been made over the past years, scientists never stop the exploration of mysteries. Experts keep examining the mechanisms of high failure rate of pulmonary grafts in mouse, further study ex vivo lung perfusion (EVLP) and so on. In this book, several articles were included to give a review on these technical problems.

Hopefully this book will be helpful for lung transplant surgeons and we looking forward to having more innovations in the second edition of the book in the future!

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Over the last 55 years lung transplantation has evolved from an experimental procedure to established therapy for many patients suffering from end stage pulmonary failure. Approximately 4000 lung transplants are being performed annually with many institutions around the world opening new programs to meet their local need. Despite its many successes, the field of lung transplantation faces many challenges. These include a relative shortage of donor organs and persistently inferior long-term outcomes compared to other solid organs.

This book provides state of the art reviews and perspectives from leading groups on topics that are highly relevant to lung transplantation, both clinically and experimentally. Surgeons from Duke University, the site of one of the largest and most experienced lung transplant programs in the world, review their institutional experience and also discuss the expanding use of extracorporeal membrane oxygenation in the care of lung transplant patients. The lung transplant group at The Ohio State University provides an overview of indications and outcomes for pediatric and adult lung transplantation. Along these lines, physicians from The Prince Charles Hospital in Brisbane describe therapeutic options for patients suffering from COPD, which continues to be one of the most common indications for lung transplantation. The Toronto lung transplant group reviews ex vivo lung perfusion, which has been one of the most important advances in this field in the recent past. Not only has the Toronto lung transplant group established that this technique allows for an evaluation and optimization of potentially marginal donor lungs, but, perhaps more importantly, ex vivo lung perfusion will hopefully provide a platform to target cellular and molecular pathways that are potentially deleterious. Undoubtedly, ex vivo lung perfusion has contributed to a reevaluation of lung donor selection criteria, a topic that is reviewed by surgeons from the University of Pennsylvania and The University of Louisville. Animal studies have paved the way for the first attempts at human lung transplantation and have played an instrumental role in refining and perfecting surgical techniques. Technical aspects of bilateral sequential lung transplantation are reviewed by the group at The University of Pittsburgh and techniques for airway anastomoses, widely perceived to be a barrier to successful lung transplantation in the early days, are reviewed by the thoracic surgical group at The University of Rome, which is renowned for their work in airway reconstructions. Advances in lung transplantation and improvements in long term survival will depend on gaining a better understanding of the unique nature of immune responses to pulmonary grafts. Pre-clinical murine models of lung transplantation enable investigators to design experiments that shed mechanistic insight into pathways that contribute to primary graft dysfunction, acute rejection and chronic lung allograft dysfunction. To this end, the group at Washington University in St. Louis summarizes their experience with orthotopic mouse lung transplantation and also describes technical aspects of lung re-transplantation in the mouse. Studies in these models have already suggested that immunosuppressive strategies that are currently used for lung transplant recipients, reviewed by the group at Columbia University, may not be optimal or even be deleterious for these patients. This is in large part due to the fact that immunosuppression used for lung recipients has been designed based on the experience with recipients of other grafts such as kidneys and livers without taking organ-specific differences in immune responses into account.

Thus, this book represents a collection of contemporary topics that are highly pertinent for health care professionals, who are involved with the care of lung transplant patients, as well as for investigators, who are engaged in the study of pulmonary transplantation biology. Lung transplantation has come a long way. Undoubtedly, new discoveries regarding the biology of lung transplantation will ultimately result in personalized treatment approaches and improved outcomes for our patients.

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Lung Transplantation (LT) has enjoyed tremendous success and it is now a well-established live-saving procedure for patients suffering from end-stage lung diseases. Since the inception of clinical LT by Dr. Cooper and colleagues in Toronto in 1983 (first successful single lung transplant) and in 1986 (first successful double lung transplant) many advances have occurred in the field–thanks to many efforts from the transplant research community and braveness of patients undergoing novel high-risk procedures and treatments. Due to these advances, this highly complex procedure became very safe and in most centers early mortality is rare, and 1 year and 5-year survival have reached 90% and 60% respectively. However, two problems still challenge the full success of LT. First, the discrepancy between donor supply and wait list continues to widen. This results in a persisting large numbers of patients with chronic lung diseases not having access to live saving transplantation. This book addresses this problem by (I) discussing proper criteria for listing patients to LT, (II) using novel extra-corporeal technologies to maintain these patients alive until an organ becomes available, and (III) by creating strategies to significantly increase donor organ availability via normothermic ex vivo lung perfusion. Secondly, chronic lung allograft dysfunction (CLAD) is unfortunately a very common occurrence after LT and a major responsible why almost half of the patients either die or require a second transplant by 5 years after the procedure. Again, this book nicely addresses state of the art clinical strategies to prevent and treat CLAD, and importantly the recent development of experimental models of CLAD in murine transplantation. These models now open much opportunity not only for better understanding of pathways and mechanisms leading to CLAD but also to study efficacy of new interventions for this disease.

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Lung transplantation exchanges one set of medical conditions for another. For patients with end-stage lung disease, lung transplantation is the only viable long-term solution that improves the quality of and extends the quantity of life. Exchanging the insidious diseases that suffocate patients and curb all their quality of life for a different set of chronic medical conditions is a choice that most patients with end-stage lung disease are willing to make.

Even though lung transplantation has been evolving for over a half-century, it's only the most recent decade or so we have seen accelerated advances in our outcomes, surgical technique, and medical management. Our understanding of the surgical science has allowed us to refine our techniques to minimize perioperative complications while at the same time our modulation of the immune response and chronic allograft dysfunction have enable the long-term success of our recipients.

Perhaps most excitedly, we have seen the introduction and refinement of *ex vivo lung perfusion* (EVLP). This technology and this time in transplantation is perhaps the most exciting and potentially revolutionary for our field since the advent of cyclosporine. We have seen rapid growth in perfusion technology where the ability to assess, repair, and modify organs is being undertaken. Transplant centers, both academic and private, as well as industry, are making huge advances and our ability to recover marginal organs and assess quality and other marginal allografts that we would not have considered just years ago. As this expertise grows to other organ systems we will no doubt see a growth in multiorgan assessment and repair centers.

This book is a composite of leading articles on lung transplantation by the worldwide leaders in lung transplantation. This book has been assembled in such a fashion as to be able to provide a depth and breadth of content for the thoracic transplantation community. The information is intended to provide context for the current state-of-the-art, recipient and donor selection and management, and exciting clinical, translational, and basic science advances that are at our fingertips.

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Lung transplantation at Duke

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Abstract: Lung transplantation represents the gold-standard therapy for patients with end-stage lung disease. Utilization of this therapy continues to rise. The Lung Transplant Program at Duke University Medical Center was established in 1992, and since that time has grown to one of the highest volume centers in the world. The program to date has performed over 1,600 lung transplants. This report represents an up-to-date review of the practice and management strategies employed for safe and effective lung transplantation at our center. Specific attention is paid to the evaluation of candidacy for lung transplantation, donor selection, surgical approach, and postoperative management. These evidence-based strategies form the foundation of the clinical transplantation program at Duke.

Keywords: Lung transplantation; transplant evaluation; Duke University

Submitted Jan 27, 2016. Accepted for publication Feb 11, 2016.
doi: 10.21037/jtd.2016.02.08
View this article at: http://dx.doi.org/10.21037/jtd.2016.02.08

Lung transplantation remains the only potentially life-saving therapy for many individuals with end-stage lung disease. Utilization has continued to rise over the past 20 years. Most recently, the ISHLT registry reports that over 4,000 adult and pediatric lung transplants were performed internationally in 2013 (1).

Established in 1992, the lung transplant program at Duke University Medical Center remains one of the largest volume lung transplant centers in the world. Since its inception, our program has performed more than 1,600 lung transplants. We have previously reported our experience in the first 15 years of the program (2). This report describes more recent practice and management strategies, as well as a reflection upon the impact of the first ten years of the lung allocation score (LAS) in the U.S..

Since the implementation of the LAS in May of 2005, the lung transplant volume experience at Duke has grown and included 1,059 transplant procedures. This includes multi-organ transplants such as heart-lung, lung-liver, lung-kidney, heart-lung-liver, and lung-bone marrow transplants. *Table 1* provides demographic data for lung only transplants performed at Duke University Medical Center following the implementation of the LAS.

Transplant candidacy

The ISHLT recently updated its guidelines for selection criteria for lung transplant (1). In an attempt to balance the scarcity of donors and maximize societal benefit of lung transplantation, the indications for lung transplant have been updated to denote greater attention paid to the potential life years gained. It is now recommended that lung transplant only be considered in patients with >50% risk of death from lung disease within two years without transplant, >80% chance of 90-day survival after transplant and >80% expected 5-year survival with transplant from general medical perspective, provided adequate graft function.

The broadness of this document reflects our practice of considering the candidacy of every patient with end-stage lung disease. While numerous relative contraindications to

Table 1 Lung transplant demographic data following implementation of the lung allocation score at Duke including 1,059 lung transplant procedures

Demographics	N	%
Lung allocation score, median (IQR)	41.86 (16.72)	
First transplant	992 (94%)	
Second transplant	67 (6%)	
Male	652 (62%)	
Bilateral	788 (74%)	
Single	243 (26%)	
Recipient age, median	60	
Recipient age, range	15 (min), 77 (max)	
Native lung disease		
Bronchiectasis	18	1.7
Bonchiolitis obliterans	16	1.5
Obstructive lung disease	221	21.4
Cystic fibrosis	161	15.6
Congenital vascular	7	0.7
Bronchoalveolar carcinoma	2	0.2
Interstitial lung disease	545	52.8
Sarcoidosis	39	3.8
Primary graft dysfunction	2	0.2
Pulmonary hypertension	22	2.1
Other	26	2.5

LAS, lung allocation score.

transplantation may be present, a holistic risk assessment of each patient's medical comorbidities, functional status, psychosocial milieu, and potential life expectancy with transplant opens up the possibility of lung transplantation to many who otherwise may not have previously been offered this therapy.

This strategy has led to our experience with multi-organ transplant combinations in those with severe multi-organ dysfunction. We will also offer combined lung transplant and cardiac surgery, including coronary artery bypass grafting (CABG), valve repair and complex vascular reconstructions to carefully selected patients. Our center has successfully bridged many critically ill patients to transplant using extracorporeal membrane oxygenation (ECMO). We have also performed successful staged lung—hematopoietic stem cell transplants in individuals with severe immunodeficiency syndromes with a goal of both allograft tolerance and cure of underlying immunodeficiency. Some exceptional circumstances, such as high degree of HLA sensitization, may require living lobar donation for lung transplantation as well.

Transplant evaluation

Our standard evaluation includes a multidisciplinary approach defined by pulmonary, cardiothoracic surgery, transplant psychology, social work, physical therapy and financial consultations. A nutritionist screens all candidates and performs a nutritional assessment in all patients with cystic fibrosis as well as those felt to be at increased risk for malnutrition. A clinical pharmacist meets with each patient for medication review and education prior to listing.

Based on data indicating an association between pre-transplant physical fitness and improved post-transplant survival, we require all patients who are able to participate in pre-transplant physical therapy (3,4). Our rigorous program maximizes respiratory muscle strength as well as total body conditioning in preparation for surgery. Physical requirements and exercise plan prior to transplantation are outlined in *Table 2*. We require candidates to walk at least 1,000 feet in 6 minutes (without limitation on oxygen usage) as well as 1/2 miles in 20 minutes on a track using as much oxygen as is necessary to maintain oxygen saturations >88%. While level surface walking is the most important component of the physical therapy program, it also includes stationary bike and strengthening, stretching and diaphragmatic breathing exercises. Included in the physical therapy program are educational classes intended to prepare both the candidate and his or her caregivers for routine post-transplant care as well as anticipatory guidance for common complications they might expect. These classes include teaching directed at self-monitoring of vital signs and home spirometry, transplant medications, diabetes management, managing a feeding tube and coping skills training.

Required pulmonary testing includes full pulmonary function testing, an arterial blood gas on room air, PA and lateral chest X-ray, a 6-minute walk test, non-contrast chest CT scan, quantitative ventilation and perfusion scan, and fluoroscopy of the diaphragms. Abnormal results will prompt additional testing. For instance, if abnormalities in swallowing function are identified on barium swallow, we perform functional endoscopic evaluation of swallowing (FEES) testing. Impaired diaphragm function may prompt maximal inspiratory and expiratory pressure measurements (MIP/MEP) with pulmonary function testing.

Cardiac evaluation includes an electrocardiogram,

Table 2 Physical requirements prior to lung transplantation at Duke University

Physical requirements prior to lung transplant surgery
Walking
Ambulate 30 minutes. (5–7 days every week)
No restriction on ambulatory assistive devices (walker, cane, etc.)
No limit on the amount of oxygen used; however, you must roll or carry your own oxygen source
No rest stops
Distance covered should be at least 2/3 mile
Bike
Bike on any type of manual bicycle (standard stationery bicycle, Airdyne, or recumbent bike)
Bike at least 20 minutes
No rest stops
No limit on the amount of oxygen used
Bike load must be at least 0.5 kp
Treadmill
2.0 mph
No rest stops
No limit on the amount of oxygen used
30 minutes
Strength training and flexibility
Use dumbbells, cuff weights, therabands, or weight training equipment (i.e., Cybex) to strengthen upper and lower extremities and trunk
Perform stretching exercises of all major muscle groups of the trunk and extremities
Education
Breathing exercises—the patient will be able to perform diaphragmatic and pursed lip breathing independently and paced breathing with exercises
The patient will have a thorough understanding of medications that may be used post-operatively
The patient has been informed of the rehabilitation requirements post-operatively and has a plan for meeting those requirements
The patient will have realistic expectations of the outcomes following transplant surgery
These are minimum requirements
**Cessation of all nicotine and tobacco products is mandatory, e.g., cigarettes, cigars, chewing tobacco and nicotine gum

right heart catheterization and echocardiogram with bubble study on all patients. Those over the age of 40 undergo left heart catheterization or CT coronary angiography. If significant cardiac disease is found, intervention and a follow up stress test may be required. For good risk patients (e.g., less than 65 years old, high functional status, etc.) concomitant revascularization via CABG and lung transplant can be considered if necessary. For higher risk patients, pre-transplant percutaneous coronary revascularization via stenting is preferred. Drug eluting stents are avoided during the evaluation period because of the need to be off dual antiplatelet platelet therapy around the time of transplant. If it is anticipated a patient will require intra-aortic balloon pump support at the time of transplant, we will obtain a reconstructed CT angiogram of the abdomen and pelvis for vascular access planning. Patients with underlying sarcoidosis undergo cardiac MRI test to look for evidence of sarcoid infiltration of the heart. If significant involvement is seen, patients are considered for heart/lung transplant.

Our gastrointestinal evaluation includes a barium swallow, 24-hour pH probe testing, and esophageal

manometry. Solid gastric emptying testing is performed when there is concern for gastroparesis. Imaging of the liver is also required to screen for cirrhosis. This is done with ultrasound in patients under the age of 55 and a CT scan in those over the age of 55. Patients with suspicious imaging, laboratory results, or clinical history may need liver fibroscan and/or biopsy with portal venous pressure gradient monitoring for additional investigations.

Lab testing includes routine hematologic, chemistry and coagulation studies as well as viral serologies for cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus (EBV), and varicella zoster virus (VZV). We also screen for the indolent chronic infections such as syphilis, hepatitis B, C and HIV. Positive screening tests prompt polymerase chain reaction (PCR) testing and would require treatment prior to proceeding with transplant. Routine, age-appropriate cancer screening as recommended by the US Preventive Services Task Force (USPSTF) is required on all patients. We also perform serologic screening for malignancy using tumor markers, including prostate specific antigen (PSA), carcinoembryonic antigen (CEA), beta human chorionic gonadohormone (β-HCG) and follicle stimulating hormone (FSH). Positive results prompt more invasive testing. The identification of low-grade, indolent malignancies within two years of transplant listing requires specialty consultation, but is not necessarily considered an absolute contraindication to transplant at our center. These include Gleason stage 6 or less prostate cancer as well as localized non-melanoma skin cancers. Additionally, we will consider transplant in patients with stage 1 non-small cell lung cancer when the transplant would be curative treatment for disease.

Data suggest that patients with idiopathic pulmonary fibrosis related to telomerase mutations are at increased risk of bone marrow and renal failure after lung transplantation (5-7). Because of this, we have recently begun evaluating telomere length in patients with familial or early onset pulmonary fibrosis, premature greying of the hair and leukopenia or thrombocytopenia. If telomere length is confirmed to be in the bottom decile, we will trial the patient on immunosuppressives prior to transplant listing to ensure they are tolerant of therapies.

Management of HLA antibodies

All candidates are screened for the presence of HLA antibodies using flow cytometry. A positive result will prompt specificity testing using Luminex single antigen bead testing. Our lab uses a mean fluorescence intensity (MFI) cutoff of 1,000 to be considered a positive result. All circulating HLA antibodies are considered unacceptable antigens and are avoided by means of a virtual crossmatch in all patients with calculated panel reactive antibody (cPRA) of less than 25%. For patients with a cPRA of greater than 25%, the HLA lab will generate a chart with all antibodies and their intensity over time for all patients prior to listing. The transplant physicians then, in consultation with the HLA lab, determine which antibodies are most likely to result in a positive crossmatch. Generally, this includes all antibodies present at 1:16 dilution and those with MFI >4,000. These HLA antibodies are all considered unacceptable antigens and avoided by means of a virtual crossmatch. Antibodies with less intensity or antigens with less cell surface expression that are thought to be possibly non-specific or not clinically significant are included on the waitlist and considered unacceptable on virtual crossmatch. However, if a donor is available and a prospective crossmatch can be performed and is negative, that organ may be accepted for that recipient. All patients with a cPRA ≥25% are treated with intravenous immunoglobulin (IVIG) intraoperatively at 2 grams/kilogram dose.

On some occasions, we have accepted a donor for a highly sensitized recipient who is clinically deteriorating when there is a positive virtual crossmatch for an antigen that is of questionable intensity, and a prospective crossmatch is not possible. Plasmapheresis is performed intraoperatively and rabbit antithymocyte globulin (rATG, 3 g/kg dose) is used for induction instead of basiliximab. Patients then receive rituximab 1 gram IV on post-operative day 1. Further plasmapheresis and antibody-directed therapy may be continued depending on the results of the retrospective crossmatch. Another option in this scenario would be to perform ex-vivo perfusion of the lungs in order to delay transplant until a negative crossmatch is confirmed.

Donor selection and management

Aggressive potential donor evaluation and management, paired with prudent selection of donors, has at our center led to minimal wait list mortality without adversely impacting short- or long-term outcomes following transplantation. In particular, appropriate donor management is critical to the optimization of potential allografts.

International guidelines from the ISHLT inform rough criteria with which to evaluate a potential donor. Our group has demonstrated that the donor pool may be safely

increased through the careful selection of donors outside the concept of an "ideal" donor as described by early international guidelines. We evaluate donors over the age of 55, as well as organs that may require periods of cold ischemia greater than 6 hours, as the more conservative measures excluding those donors do not result in improved outcomes in the available published evidence (8).

Frequently, donor management during the period of evaluation may not reflect the optimum strategy for lung preservation. In particular, low volume and low pressure ventilatory settings may lead to donor lung atelectasis that manifests as inadequate gas exchange and abnormal chest roentogram. Low PaO_2/FiO_2 ratios (less than 300) may frequently be due to reversible conditions such as atelectasis, pulmonary edema, or mucous plugging. With appropriate recruitment of the donor lungs and pulmonary secretion clearance, significant improvement in gas exchange is achievable and excellent post-transplant outcomes may be attained with organs initially felt to be unsuitable for transplantation. In addition to recruitment maneuvers to improve oxygenation, the donor's hemodynamics and physiology should be optimized. Hemodynamic stability should be achieved with minimal use of inotropic support. When needed, vasopressin arginine may support blood pressure and permit diuresis to optimize donor fluid balance and acid-base status. The use of a pulmonary artery catheter is frequently advised to permit goal-directed therapies for the attainment of appropriate loading conditions and optimum volume status. Published data from randomized trials demonstrate that a judicious use of diuretics, conservative fluid management, and protective ventilator protocols for donors leads to improved lung allograft utility, without adversely affecting other organ function (9).

Although donor cultures from bronchoalveolar lavage are important for appropriate tailoring of post-transplant antibiotics, rarely should donor microbiologic results prohibit or dissuade use of the allograft for transplantation. Culture-directed antibiotic therapy successfully prevents fatal complications in the event of donor to recipient transmission of infectious organisms (10). However, potential donors colonized with Genomovar 3 Burkholderia cenocepacia or other highly virulent, multi-drug resistant organisms may need to be excluded from donation on account of extremely complex resistance patterns (11).

The candidate donor should be size-matched to the anticipated recipient. In our practice, horizontal and vertical measurements based on plain chest radiographs are used to assist with appropriate matching. The predicted total lung capacity of recipients and donors can also be calculated using standard formulation. Extreme size mismatch, either too large or too small, confers a survival disadvantage in published series (12,13). In the event of a large donor matched to a smaller recipient, lung reduction can be performed at the time of transplantation. Our preferred method of pneumoreduction is anatomic resection of the right middle lobe and/or lingulectomy. Very rarely cadaveric lobar transplantation can be performed; however, this appears to increase the perioperative risks of transplantation to some degree (14).

Ex vivo lung perfusion (EVLP) is deployed clinically in selected donors as a means to further interrogate allograft function prior to committing to transplant. Although it may possibly improve the quality of an otherwise marginal candidate allograft, current EVLP technologies permits further assessment of the graft prior to proceeding with transplantation. Candidate grafts in which the suitability for transplantation is uncertain may be serially assessed on the EVLP device during a period of optimum ventilator management in order to ascertain if the graft is appropriate for transplantation. We use clinically the XVIVO Perfusion System (XPS™) as was used in the NOVEL clinical trial. This is currently the only device for EVLP approved for use in the United States by the FDA. In our experience, nearly half of the allografts evaluated on the XPS system were subsequently used for transplantation. We have transplanted 20 recipients thus far using this device, with short and long terms outcomes no different from our standard lung donor cohort. The final results of the trial have yet to be published and the sponsor is actively accruing additional patients for an extension of the trial.

Surgical approach

As introduced previously, every attempt is made to match the candidate recipient with the optimum procedure. Comprehensive evaluation identifies the appropriateness of the available therapies. Single-lung, bilateral-lung, bilateral lobar, heart-lung, and lung with concomitant cardiac surgery are all available therapies based on pre-transplant evaluation.

For the majority of patients, bilateral orthotopic lung transplantation (BOLT) is the preferred procedure. Patients receiving bilateral allografts enjoy improved long-term survival and a lower rate of chronic allograft dysfunction (15). Septic lung disease such as cystic fibrosis mandates BOLT, as does severe pulmonary hypertension. In the case of

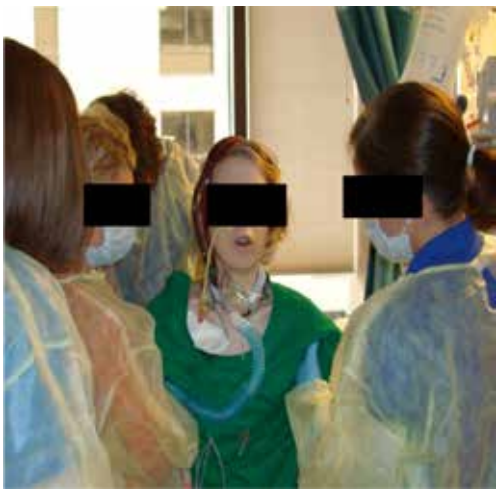

Figure 1 A patient ambulates with assistance while supported by VV ECMO deployed percutaneously through a dual-lumen cannula in the right internal jugular vein. VV, veno-venous; ECMO, extracorporeal membrane oxygenation.

interstitial lung disease, we do stratify patients based on age, functional status, and other comorbidities. We pursue BOLT for lower risk patients and single orthotopic lung transplantation (SOLT) for patients thought to be at a high perioperative risk. We generally consider higher risk patients to be those over age 65, with coronary disease, marginal renal function, or increased frailty. Data suggest that in older patients with idiopathic pulmonary fibrosis (IPF), the long-term benefits of BOLT may not be fully realized due to increased perioperative risk (16). In very select circumstances we have considered staging a bilateral procedure by performing two single lung transplants at discrete time points. We have done this electively in 12 recipients. Results suggest similar perioperative outcomes except for diminished rates of renal dysfunction in the staged BOLT approach. Long-term benefit of the staged approached is still under investigation.

For single lung transplantation, an anterolateral thoracotomy incision in the 4th or 5th intercostal space permits excellent exposure for the transplant procedure. This can also be done via a posterolateral approach if the surgeon prefers. For double lung transplantation, we prefer a clamshell incision by way of bilateral anterolateral thoracotomies in the 4th intercostal space, in conjunction with a transverse sternotomy. The clamshell incision yields generous exposure and can facilitate rapid deployment of cardiopulmonary bypass or ECMO if needed. In patients with a planned concomitant cardiac procedure that mandates cardiopulmonary bypass (CPB), a median sternotomy may be optimal if pleural adhesions are thought to be minimal. Our preference is to avoid CPB if possible as it has been associated with increased rates of primary graft dysfunction (PGD) and transfusion requirements. Mechanical support intraoperatively should be tailored to the needs of the present scenario; however, ECMO remains our preferred support method if full bypass is not required. Intra-aortic balloon pump (IABP) is a useful adjunct for patients with depressed left ventricular (LV) function and those with coronary artery disease.

Though our evaluation and listing process aims to identify patients sick enough to benefit from transplantation but otherwise healthy enough to tolerate the procedure, a small subset of our patient population progresses to respiratory failure pre-transplant. In highly selected patients, we provide ECMO support as a bridge to transplantation. In this setting, our most common practice is to support these patients with veno-venous (VV) ECMO through a percutaneously inserted Avalon catheter into the right internal jugular vein, then initiate pre-transplant active rehabilitation as a means to recover the debilitated patient prior to transplant. Nutritional support is via a gastrojejunostomy tube and sedation and ventilator support are weaned as low as possible or off as tolerated once ECMO is initiated. Active rehabilitation while on ECMO includes passive resistance exercises, as well as ambulation. *Figure 1* depicts a patient ambulating while supported by VV-ECMO. Our early experience included patients supported in this manner with VV-ECMO as a bridge to transplantation, with 100% survival to one year. Patients able to ambulate and participate in physical therapy while supported by VV-ECMO pre-transplant demonstrated significantly shorter times to extubation, shorter ICU stays, and shorter index hospitalizations. Economic analysis suggests that these benefits associated with ambulatory ECMO lead to decreased total cost of index hospitalization associated with lung transplantation (17-19). Veno-arterial (VA) ECMO may be necessary in patients with severe PH and RV failure who require mechanical support prior to bridging. Utilizing an axillary arterial and right IJ cannulation strategy, our strategy of active rehab while on ECMO can still be attained. The transplant procedure may be conducted while on ECMO support, or transitioned to cardiopulmonary bypass if required.

Duke has made additional contributions to the field of

lung transplant in pioneering novel procedures to treat complex vascular abnormalities that might otherwise pose a contraindication to transplantation. We described the use of simultaneous lung and RVOT allograft as a means to treat aneurysmal disease of the pulmonary artery at the time of lung transplant (20). Transplantation of the RVOT avoids the need for concomitant heart transplant or the need for a complex repair with prosthetic material to treat a pulmonary artery aneurysm. This approach has been utilized in both single and bilateral lung transplant procedures, as well as in the setting of reoperation after a remote correction of tetralogy of Fallot led to pseudoaneurysm of the RVOT. In each setting, the use of RVOT allograft can minimize morbidity and permit transplantation in patients who might otherwise be turned down for the procedure. If the RVOT allograft is not available, then at times a homograft has been utilized with good success.

Primary graft dysfunction (PGD)

PGD after lung transplantation remains a significant source of early morbidity and mortality. Patients surviving PGD are also at risk for long-term alloimmune consequences and decreased overall survival, suggesting a link between PGD and subsequent development of BOS. Prompt diagnostic workup is mandatory to evaluate for alternative causes of respiratory failure, to include vascular torsion, infection, cardiogenic edema, or hyperacute rejection. During the transplant procedure, several steps are taken at our center to minimize the extent of reperfusion injury experienced by the allograft. In addition to the use of extracellular preservation solutions, we administer intravenous methylprednisolone (500 mg) and mannitol (25 mg) prior to reperfusion of both allografts. Importantly, reperfusion is performed in a controlled fashion over a period of 10-15 minutes. Similarly, ventilation and lung recruitment should be held until the newly implanted lung has rewarmed. Inhaled nitric oxide (iNO) is used to decrease pulmonary vascular resistance during the operation. If additional pulmonary vasodilation is thought to be necessary, the patient can be weaned from iNO to inhaled epoprostenol (Veletri) after initial stabilization in the intensive care unit and prior to extubation.

Those patients exhibiting PGD despite preventive measures are considered for ECMO support. Those with peak inspiratory pressures approaching 30 cm H_2O and requiring FiO_2 greater than 0.60 after excluding other causes for failure are considered candidates for post-transplant ECMO. VV ECMO provides short-term support while lung recovery is anticipated. Since 2001, approximately 5% of lung transplant recipients at our center have required VV-ECMO support for primary graft dysfunction following transplant. Support can be initiated at the bedside by way of a single dual-lumen cannula in the right internal jugular (RIJ) vein. In consultation with our anesthesia teams, our preference is to place central lines in the left internal jugular vein pre-transplant in order to more easily facilitate initiation of ECMO by way of the RIJ if needed post-transplant. Once ECMO support is established, patients are transitioned to lung-protective ventilatory settings with low pressures and FiO_2 of 0.21. Of those patients requiring VV-ECMO post-transplant at our center, over 95% are successfully weaned from support as their graft performance improves. Patients are typically weaned from ECMO within 24–72 hours as evidence of pulmonary recovery is observed. Though survival rates of those experiencing PGD continue to improve with advances in ECMO technology, PGD continues to decrease overall survival rates and leads to a decrease in overall graft function once free from ECMO support (21-23).

Immunosuppression

Our standard immunosuppression regimen consists of basiliximab for induction and tacrolimus, prednisone and mycophenolate mofetil for maintenance immunosuppression. Basiliximab 20 mg is administered intraoperatively and again on postoperative day 4. Intraoperatively we also administer 500 mg IV methylprednisolone at the time of each allograft reperfusion and mycophenolate mofetil 1,000 mg intravenously once. The recipient starts tacrolimus prior to the transplant, at the time the donor lungs are deemed acceptable and the decision to proceed with transplant is made, with a single dose of 1 mg tacrolimus sublingual (0.5 mg for patients >age 65 or on a triazole antifungal).

Tacrolimus troughs are measured starting post-operative day 2. We typically target tacrolimus trough levels 12–15 mcg/L in the first year, with lower target troughs in patients over the age of 65 or with significant renal dysfunction. Target troughs are generally decreased over time depending on rejection episodes and renal function. Patients are given methylprednisolone 125 mg IV q12h ×4 doses and then maintained on prednisone 20 mg daily for the first three months. Prednisone is typically tapered in 5 mg increments every three months until a basal dose of 5 mg daily is reached. Mycophenolate is continued at 1,000 mg

twice daily, with discontinuation or dose reductions in the setting of leukopenia or severe infectious complications.

All patients with a cPRA ≥25% are treated with intravenous immunoglobulin (IVIG) intraoperatively as stated previously. IVIG is continued weekly for six weeks after the transplant, then monthly for three months and then every three months for the first year after transplant. If the HLA antibody screen is negative on two samples, IVIG is discontinued.

Infection prophylaxis

Standard intraoperative antibiotic prophylaxis includes cefepime for gram negative coverage, vancomycin for gram positive coverage and fluconazole for candida prophylaxis. The cefepime is typically discontinued after 7–10 days once all intraoperative cultures are finalized as negative. Vancomycin is generally continued for the duration of chest tubes being in place. We have recently begun extending fluconazole duration for 90 days after the transplant to decrease risk of invasive candidiasis. We also use inhaled liposomal amphotericin for additional fungal prophylaxis to target airway mold colonization. This starts POD 1, and continues daily ×4 days prior to going to weekly for the duration of the transplant hospitalization. Patients with known pretransplant colonization with antimicrobial pathogens, such as those with cystic fibrosis, are evaluated by transplant infectious disease for development of a customized perioperative antibiotic regimen. We typically continue pathogen-directed antimicrobials for a minimum of 14 days post transplant.

We use sulfamethoxazole/trimethoprim 80/360 mg daily as our first line agent for pneumocystis jeroveci prophylaxis starting seven days after the transplant and continuing indefinitely. Inhaled pentamidine, dapsone and atovaquone are second line agents used in those with intolerance to sulfamethoxazole/trimethoprim. Patients take nystatin swish and swallow four times daily for the first six months post transplant for oral candida prophylaxis.

Our viral prophylaxis protocol is dependent on donor and recipient CMV status. Recipients who are at risk for CMV going into transplant (either recipient CMV IgG positive or donor CMV IgM/IgG positive) are treated initially with ganciclovir 5 mg/kg IV q24h and transitioned to valganciclovir 900 mg PO daily. Recipients with prior exposure to CMV going into transplant are continued on CMV prophylaxis for 12 months following transplant. Those who are high risk for CMV disease due to donor CMV IgG positivity without pre-transplant recipient exposure, i.e., CMV recipient IgG negative, are continued on prophylaxis indefinitely as tolerated. Gancilcovir and valganciclovir dosing is adjusted based on renal function. In patients who are both donor and recipient CMV IgG negative, acyclovir prophylaxis is given IV initially and then at a dose of 400 mg PO bid for the first six months after transplant.

Post-transplant monitoring

After discharge from the transplant hospitalization, patients return to lung transplant physical therapy for reconditioning and strength training. All are required to complete a minimum of 23 sessions. They are followed in the transplant pulmonary clinic on a weekly basis during this time. We monitor radiographic imaging, spirometry, blood gases, immunosuppressive drug levels and routine labs.

In the first year after the transplant we perform regularly scheduled surveillance bronchoscopies with bronchoalveolar lavage and transbronchial lung biopsy at 2–4 weeks, 3, 6, 9 and 12 months after the transplant. Bronchoscopies are also performed as clinically indicated (new respiratory symptoms, radiographic abnormalities, drop in lung function). We monitor for CMV with serum PCR testing and the development of HLA antibodies at the time of routine surveillance bronchoscopies. If acute rejection is found, two follow-up bronchoscopies are performed at 4-6-week intervals after treatment to ensure the rejection has been effectively managed. Thereafter, we generally perform an annual bronchoscopy long term as a screening for indolent rejection and infection.

The bronchoalveolar lavage return is sent for cell differential, bacterial, fungal, mycobacterial culture and an extended respiratory viral PCR. The viral PCR analyzes for the presence of influenza, RSV, adenovirus, parainfluenza, human metapneumovirus and rhinovirus. Patients with a neutrophilic-predominant cell differential on bronchoalveolar lavage are considered for treatment with azithromycin 250 mg PO three times weekly.

Management of rejection

Our standard, first line treatment of acute cellular rejection is with corticosteroids. We use methylprednisolone 10 mg/kg IV daily (rounded to the nearest 250 mg) ×3 days followed by a taper of prednisone starting at 60 mg daily and

Table 3 Antibody mediated rejection protocol

Treatment of antibody-mediated rejection				
Day 1	Plasmapheresis (1.0 PV with albumin replacement)	Methylprednisolone 500 mg IV	Bortezomib 1.3 mg/m^2 SQ	Rituximab 375 mg/m^2 IV
Day 2		Methylprednisolone 500 mg IV		
Day 3		Methylprednisolone 500 mg IV		
Day 4	Plasmapheresis	Methylprednisolone 50 mg IV	Bortezomib 1.3 mg/m^2 SQ	
Day 8	Plasmapheresis	Methylprednisolone 50 mg IV	Bortezomib 1.3 mg/m^2 SQ	
Day 11	Plasmapheresis	Methylprednisolone 50 mg IV	Bortezomib 1.3 mg/m^2 SQ	Rituximab 375 mg/m^2 IV
Day 12	IVIG 500 mg/kg, continuing weekly ×4 weeks, then monthly ×3 months and then q 3 months thereafter			

decreasing by 5 mg daily until the patient reaches his or her baseline dose. The protocol for the treatment of antibody-mediated rejection is outlined in *Table 3*.

For patients with severe or refractory rejection, we treat with anti-thymocyte globulin. We typically use rabbit-derived anti-thymoglobulin at a dose of 1.5 mg/kg ×3 doses as first choice, but also use equine-derived formulations on occasion. In the setting of refractory rejection, we also evaluate for possible drivers of the rejection, such as CMV infection, inadequate calcineurin inhibitor levels, aspiration injury, medication nonadherence, community-acquired respiratory viruses and development of HLA antibodies. We consider adjusting basal immunosuppression. This may mean changing route of tacrolimus administration from PO to SL, changing from tacrolimus to cyclosporine, or an alternative to mycophenolate such as azathioprine or sirolimus.

Patients who experience either acute cellular rejection after thymoglobulin or in those with evidence of chronic lung allograft dysfunction (CLAD) are considered for alemtuzumab. This is given as a one-time, 30 mg dose. We routinely initiate extended antifungal and antiviral prophylaxis after alemtuzumab to decrease the risk of opportunistic infections. Preferred antifungal prophylaxis is posaconazole delayed release. Viral prophylaxis is dependent on the CMV status of the donor and recipient. Prophylaxis is continued until the CD4 count is greater than 100.

Because of the evidence indicating worse outcomes in patients with donor specific anti-HLA antibodies, we routinely monitor our patients for the development of anti-HLA antibodies (24,25). Patients who are highly sensitized prior to transplant or who develop new onset HLA antibodies after transplant are managed with intravenous immunoglobin (IVIG). Those who develop donor specific HLA antibodies, but do not have evidence of graft dysfunction are treated with rituximab 375 mg/m^2 IV weekly ×4 doses. This is followed by IVIG monthly for three months and then every three months for a year or until resolution of the donor specific antibodies. Rituximab is a chimeric monoclonal antibody directed against CD20 expressing cells which results in depletion of B cells. When there is a concern for antibody mediated rejection, typically based on the presence of donor specific HLA antibodies, pathologic findings and graft dysfunction, we initiate our DSA—pheresis protocol. This multimodal strategy includes plasmapheresis, high dose steroids, rituximab, bortezomib which is a proteasome inhibitor which results in plasma cell apoptosis, and IVIG (*Table 4*).

Management of gastroesophageal reflux disease (GERD)

Our program takes an aggressive approach to management of gastroesophageal reflux disease. As stated above, all patients are evaluated for reflux prior to transplant. Those with significant GERD prior to transplant (acid contact times >10% total or demeester score >20) are arranged to undergo early fundoplication after transplant, with a goal of having the procedure within the first 90 days of the transplant, depending on clinical stability and fitness for surgery. Those without significant GERD prior to transplant have repeated testing done after they are discharged from the transplant hospitalization. Studies from our center, as well as others, have shown that early fundoplication confers advantage both with respect to overall survival and in freedom from bronchiolitis obliterans (26,27).

Long-term management

While freedom from chronic allograft dysfunction decreases over time, the rates of malignancy and renal

Table 4 Inclusion and exclusion criteria for lung transplantation at Duke University

Patient selection criteria checklist for lung and heart/lung transplant candidates

Inclusion criteria

1. High (>50%) risk of death from lung disease within two years if transplant is not performed
2. High (>80%) likelihood of surviving at least 90 days after lung transplantation
3. High (>80%) likelihood of 5-year post transplant survival from general medical perspective provided there is adequate graft function
4. Patient age is >14 years of age
5. Patient participates in pre-transplant physical therapy
6. Patient is able to be at Duke Hospital within 2 hours of notification
7. Patient ability to relocate for a minimum of 23 sessions of pulmonary physical therapy after transplant
8. Presence of a consistent and reliable social support system
9. Patient has ability to meet the financial obligations projected for transplantation, immunosuppression, supportive therapies, and relocation

Additional inclusion criteria for heart/lung transplantation

The selection criteria above also apply for combined heart/lung transplant referrals. In addition, patients must have

1. Significant cardiac dysfunction that precludes isolated lung transplantation
2. Patients must be <60 years of age for consideration

Exclusion criteria

1. Patient had malignancy in the last 2 years, with case-by-case exception considered for localized malignancies with an expected 5-year survival of >80%. Patients with malignancies that are high risk for recurrence must be 5 years free of cancer
2. Patient has untreatable advanced dysfunction of another major organ system (e.g., heart, liver, kidney or brain) unless candidate for multi-organ transplant
3. Age ≥60 years old and in need of multi-organ transplant
4. Uncorrected atherosclerotic disease with suspected or confirmed end organ ischemia or dysfunction or coronary artery disease not amenable to revascularization
5. Uncorrectable bleeding diathesis
6. Chronic infection with highly virulent and/or resistant microbes that are poorly controlled pre-transplant, including genomovar 3 Burkholderia cenocepacia
7. Evidence of active Mycobacterium tuberculosis infection
8. Significant chest wall/spinal deformity expected to cause severe restriction after transplant
9. Excessive obesity or malnutrition, generally defined as <70% or >130% IBW
10. Psychiatric or psychologic condition associated with the inability to cooperate with medical/allied health team and/or adhere to complex medical therapy
11. Current or historic repeated or prolonged documented non-adherence to medical therapies and appointments
12. Substance addiction (e.g., alcohol, tobacco, narcotics, or illicit substances) that is active
13. Acute medical instability, including but not limited to sepsis, MI, liver failure
14. Severely limited functional status with poor rehabilitation potential
15. Severe or symptomatic osteoporosis
16. Chronic, active use of narcotics or benzodiazepines
17. Severe esophageal dysmotility
18. Patient chooses not to proceed with transplantation
19. Multiple co-morbid conditions that when combined make transplantation an unsafe risk
20. Current disease is too early for transplantation
21. Age ≥65 with need for concomitant cardiac surgery

dysfunction after lung transplant increase and are not inconsequential (28,29). Therefore, long term management of lung transplant recipients requires continued close monitoring of allograft function balanced against risks of immunosuppression. We generally evaluate patients every 3-4 months in lung transplant clinic for the duration of their lifespan. In addition to assessing allograft function, we screen for complications such as hypertension, hyperlipidemia, diabetes mellitus, cardiovascular disease, chronic kidney disease, hematologic disorders and malignancy routinely. We then collaborate closely with primary care providers and other subspecialists to manage these conditions and optimize outcomes for our patients.

Conclusions

Lung transplant remains an important and growing treatment option for patients with many kinds of end-stage lung disease. Since the establishment of a lung transplant program in 1992, Duke has strived for excellence in the management of patients with thoracic disease. A continuous commitment to the delivery of high-quality care has enabled Duke to meet increasing demand for this life-saving therapy. Pioneering technologies, techniques, and management strategies have enabled the program to offer this therapy to those who might previously have been denied eligibility for transplant, to rescue those who suffer graft dysfunction postoperatively, and to use early interventions to minimize post-transplant complications. In this update, we have reviewed the evidence that guides these changes in practice. Patients continue to become sicker and more complex in their comorbidities. Steady improvement in survival metrics reflects an increasing ability to safely treat these patients. More radical improvements in the field remain just ahead as we learn to take advantage of new technologies such as EVLP and novel immunosuppression. Discoveries such as these will increase the limited donor pool, allow for organ manipulation leading to improved long-term outcomes, and selectively protect the organ from immunologic injury.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Gray AL, Mulvihill MS, Hartwig MG. Lung transplantation at Duke. J Thorac Dis 2016;8(3):E185-E196. doi: 10.21037/jtd.2016.02.08

Immunosuppression in lung transplantation

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Abstract: Lung transplantation can be a life-saving procedure for those with end-stage lung diseases. Unfortunately, long term graft and patient survival are limited by both acute and chronic allograft rejection, with a median survival of just over 6 years. Immunosuppressive regimens are employed to reduce the rate of rejection, and while protocols vary from center to center, conventional maintenance therapy consists of triple drug therapy with a calcineurin inhibitor (cyclosporine or tacrolimus), antiproliferative agents [azathioprine (AZA), mycophenolate, sirolimus (srl), everolimus (evl)], and corticosteroids (CS). Roughly 50% of lung transplant centers also utilize induction therapy, with polyclonal antibody preparations [equine or rabbit anti-thymocyte globulin (ATG)], interleukin 2 receptor antagonists (IL2RAs) (daclizumab or basiliximab), or alemtuzumab. This review summarizes these agents and the data surrounding their use in lung transplantation, as well as additional common and novel therapies in lung transplantation. Despite the progression of the management of lung transplant recipients, they continue to be at high risk of treatment-related complications, and poor graft and patient survival. Randomized clinical trials are needed to allow for the development of better agents, regimens and techniques to address above mentioned issues and reduce morbidity and mortality among lung transplant recipients.

Keywords: Lung transplantation; immunosuppression; review

Submitted Feb 04, 2014. Accepted for publication Apr 16, 2014.
doi: 10.3978/j.issn.2072-1439.2014.04.23
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.04.23

Lung transplantation can be a life-saving procedure for those with end-stage lung diseases. Unfortunately, long term graft and patient survival are limited by both acute and chronic allograft rejection, with a median survival of just over 6 years (1). Immunosuppressive regimens are employed to reduce the rate of rejection, and while protocols vary from center to center, conventional maintenance therapy consists of triple drug therapy with a calcineurin inhibitor (cyclosporine or tacrolimus), antiproliferative agent [azathioprine (AZA), mycophenolate, sirolimus (srl), everolimus (evl)], and corticosteroids (CS). Roughly 50% of lung transplant centers also utilize induction therapy, with polyclonal antibody preparations [equine or rabbit anti-thymocyte globulin (ATG)], interleukin 2 receptor antagonists (IL2RAs) (daclizumab or basiliximab), or alemtuzumab (2). While these agents are used to prevent acute and chronic rejection, they are not without adverse effects, including drug-specific toxicities, as well as opportunistic infections and malignancy. This review will summarize these agents and the data surrounding their use in lung transplantation, as well as additional common and novel therapies in lung transplantation.

Induction immunosuppression

Induction therapy is intensive immunosuppressant therapy given perioperatively to reduce the risk of acute rejection and also serves to delay initiation of maintenance immunosuppression, most notably the nephrotoxic calcineurin inhibitors. These agents primarily target T lymphocytes, which are considered the effector cells in cell-mediated rejection.

According to the most recent registry report of the International Society for Heart and Lung Transplantation (ISHLT), of the centers that utilize induction, majority use an IL2RA (2). Both daclizumab and basilixmab are non-

Table 1 Induction immunosuppression

Citation	Immunosuppressant	N	Methods	Outcomes
Palmer *et al.* 1999 (7)	ATG *vs.* no induction	44	Prospective RCT	≥ A2 AR: 23% *vs.* 55%, P=0.03 BOS: 20% *vs.* 38% Survival, 1-yr: 68% *vs.* 73% Survival, 2-yr: 64% *vs.* 68% No difference in infection or malignancy
Garrity *et al.* 2001 (8)	Daclizumab *vs.* no induction	61	Retrospective	≥ A2 AR: 18% *vs.* 48%, P<0.04 No difference in infection or PTLD
Borro *et al.* 2005 (9)	Basiliximab *vs.* no induction	15	Retrospective	AR: 13% *vs.* 38.5%, P=0.19 BOS: 20% *vs.*38.5%, P=0.4 Survival, 2-yr: 80% *vs.* 54%, P=0.14 No difference in infection or malignancy
Hachem *et al.* 2005 (10)	Basiliximab *vs.* ATG	157	Retrospective	Cumulative A AR Score higher at 3-, 6-, 12-month with basiliximab, P=0.003, 0.004, 0.033 respectively BOS stage 1 at 2-yr: 36% *vs.* 26%
Burton *et al.* 2006 (11)	Daclizumab *vs.* ATG	335	Retrospective	Freedom from ≥ A2 AR, 3-month: 9% *vs.* 32% Freedom from ≥ A2 AR, 2-yr: 0% *vs.* 26% P<0.0001
Mullen *et al.* 2007 (12)	Daclizumab *vs.* ATG	50	RCT	No difference in AR or BOS at 1 year Survival: 96% *vs.* 88%
Ailawadi *et al.* 2008 (13)	Daclizumab *vs.* ATG	163	Retrospective	AR: 9% *vs.* 28%, P=0.002 BOS: 6.4% *vs.* 23%, P=0.02 Survival: 94% *vs.* 83%, P=0.05
Hartwig *et al.* 2008 (14)	ATG *vs.* no induction	44	Prospective RCT	AR: 62% *vs.* 68%, P=0.52 Early AR: 5% *vs.* 41%, P=0.01 Graft survival: 36% *vs.* 23%, P=0.048
Clinckart *et al.* 2009 (15)	Basiliximab *vs.* ATG	37	Retrospective	AR: 52.4% *vs.* 43.8%

RCT, randomized controlled trial; AR, acute rejection; BOS, bronchiolitis obliterans syndrome; PTLD, posttransplantlymphoprolifer ative disorder; ATG, anti-thymocyte globulin; yr, year.

depleting monoclonal antibodies that bind to the alpha subunit of the interleukin 2 (IL-2) receptor (CD25) present on activated T lymphocytes, thereby preventing T cell activation and proliferation (3,4). Daclizumab is a humanized (90% human, 10% murine) (3) monoclonal antibodythat was removed from the US market in 2009 (FDA), thus making basiliximab the only IL2RA available for use. Basiliximab is a chimeric (75% human, 25% murine) monoclonal antibody and is generally well tolerated, with adverse effects similar to that of placebo (4). ATG is the second most commonly used induction agent, used by roughly 20% of centers that utilize induction (2). ATG is a polyconal antibody preparation isolated from either rabbit (rATG, Thymoglobulin©) or horse (equine ATG, ATGAM©) sera which contain antibodies toward human thymocytes and cause significant T cell depletion (5,6). Adverse effects associated with these agents include fever, chills, rash, arthralgia, diarrhea, leukopenia, and thrombocytopenia. Pre-medication with acetaminophen, anti-histamines, and CS are usually required and help minimize these reactions. Serum sickness and anaphylaxis have also been reported, in addition to increased rates of infection and malignancy.

Data for the use of induction in lung transplantation are presented in *Table 1*. Overall it appears that induction with either ATG or an IL2RA reduces or delays the incidence of acute rejection, bronchiolitis obliterans syndrome (BOS), and may improve graft and patient survival compared to no induction (7-9,14). Studies comparing IL2RAs and ATG

show inconclusive results; one study indicated IL2RAs are associated with lower rates of acute rejection and BOS, as well as improved survival (13); three studies showed lower acute rejection and BOS and improved survival with ATG (10,11,15), while still another showed no difference (12). In 2008, Hachem and colleagues published a registry report that retrospectively analyzed 3,970 adult lung transplant recipients. Four year graft survival in those who received induction with an IL2RA, ATG, or no induction were 64%, 60%, and 57% (P=0.0067), respectively (16). Reasons for such variability in outcomes relate to the size and retrospective nature of these studies, potential differences in patient population and management, duration of followup, and variability in maintenance immunosuppression regimens. More recently, alemtuzumab, a humanized monoclonal antibody targeting CD52, has been used as an induction agent. The CD52 antigen is found on T and B lymphocytes, as well as natural killer cells, monocytes and macrophages (17). Upon binding, alemtuzumab induces cellular lysis and causes significant and prolonged depletion, with B cell recovery occurring within 3-6 months and T cell recovery >12 months (18,19). This profound and prolonged lymphocyte depletion associated with alemtuzumab may allow for the possibility of reduced maintenance immunosuppression. Loenhout and colleagues published their findings using alemtuzumab induction in 20 lung transplant recipients with reduced maintenance immunosuppression in 2010. Compared to 20 historical controls who received standard maintenance immunsuppression, there were no statistical differences between 6- or 12-month survival (95% *vs.* 90%, 76% *vs.* 95%), episodes of acute rejection (2/16 *vs.* 5/20), or bacterial, viral or fungal infections (20). Subsequently, Shyu and colleagues published 5 year outcomes using alemtuzumab induction with reduced-intensity maintenance immunosuppression. Their retrospective analysis grouped patients according to induction type: alemtuzumab (n=127), ATG (n=43), daclizumab (n=73), or none (n=93). Graft survival differed by group: 59%, 44%, 41%, 47%, respectively; as did freedom from acute rejection: 30%, 20%, 19%, 18%, respectively; freedom from lymphocytic bronchiolitis: 82%, 54%, 55%, 70% respectively; and freedom from BOS: 54%, 27%, 43%, 46% respectively (21). While alemtuzumab induction with reduced maintenance immunosuppression thus far demonstrates similar if not improved overall outcomes compared to other induction regimens, the optimal induction and maintenance regimen still needs to be elucidated by large, randomized controlled trials. Though 50% of centers currently utilize induction, enhanced immunosuppression must be weighed against adverse effects, including infection and malignancy. Large, randomized controlled trials measuring the difference in acute rejection, BOS, graft and patient survival, infection and malignancy comparing no induction, IL2RAs, ATG, and alemtuzumab are needed to better understand the effect of the agents and to identify the optimal regimen for lung transplant recipients.

Maintenance immunosuppression

Maintenance immunosuppression is lifelong immunosuppressive therapy that is given to prevent both acute and chronic rejection. The goal is to not only to prevent and minimize immune-mediated injury to the allograft but also to minimize adverse effects associated with the medications used. Conventional maintenance immunosuppressive regimens consist of triple drug therapy with a calcineurin inhibitor, antiproliferative agent, and CS. Historically cyclosporine and AZA were used along with prednisone, but over time additional agents have emerged on the market, including tacrolimus, mycophenolate, and the mammalian target of rapamycin (mTOR) inhibitors, srl and evl. Despite the addition of these agents to the armamentarium of immunosuppression for lung transplant recipients, acute rejection and BOS remain obstacles to long-term survival. Additionally, minimization and management of adverse effects continuesto be challenging. Selection of regimens is largely protocolized and based on studies from other types of organ transplantation as well as currently available literature in lung transplant, and center-specific outcomes and provider experience.

Calcineurin inhibitors

Cyclosporine was the first calcineurin inhibitor available for use, first approved by the FDA in 1983. It is a lipophilic compound that binds to intracellular cyclophilin in T lymphocytes, forming a complex that prevents transcription of interleukin 2, thereby decreasing activation and proliferation of T lymphocytes (22). Oral absorption of cyclosporine (Sandimmune©) is poor and variable (10-89%). A modified cyclosporine formulation was subsequently developed and approved by the FDA in 1997 (Neoral©) with enhanced bioavailability, with approximately 50-150% increases in area under the curve (AUC) and Cmax (23,24). Sandimmune and Neoral are not interchangeable but both are available in capsules, oral solution, and intravenous

formulations. Therapeutic drug monitoring of cyclosporine consists of measuring trough (C0) values, AUC calculations, or 2-hour post-dose (C2) levels. In renal transplantation, AUC measurements have demonstrated superiority over troughs (25), however this requires multiple samples to estimate AUC, which is time consuming, cumbersome and impractical. A limited sampling strategy (LSS) may be employed as an alternative, measuring 2 post-dose levels (26), but this method still requires multiple samples and a calculation to estimate AUC. Therefore most centers utilize either C0 or C2 levels. Studies in lung transplant recipients indicate that C2 is a better correlate with AUC than C0 (27) and may reduce short-term nephrotoxicity associated with cyclosporine compared with C0, without compromising lung function (28). Target ranges vary according to center-specific protocols and practices, and take into account patient characteristics, such as time post-transplant and rejection and infection history. Generally, target trough levels range from 100-450 ng/mL, or C2 levels 800-1,400 ng/mL. Major adverse effects of cyclosporine include nephrotoxicity (acute and chronic), hypertension, hypercholesterolemia, electrolyte abnormalities (hyperkalemia, hypomagnesemia), neurotoxicity (posterior reversible encephalopathic syndrome, seizures, headache, tremor), diabetes, hirsutism, and gingival hyperplasia. A second calcineurin inhibitor, tacrolimus(previously known as FK506) (Prograf©) became available for use in 1997. It is 10-100 times more potent than cyclosporine. Tacrolimus binds to intracellular FKBP12, forming a complex that prevents transcription of cytokines, including interleukin 2, and ultimately prevents T lymphocyte activation and proliferation (29). Like cyclosporine, tacrolimus has poor and variable absorption, 17-23% (29). Tacrolimus is available in oral capsules and as an intravenous formulation. There is no commercially available oral suspension however formulas for pharmaceutical compounding are available. Sublingual administration of tacrolimus capsules at half of the oral dose is an option for those who are unable to tolerate oral therapy and wish to avoid intravenous tacrolimus due to significant toxicity (30). A once-daily extended-release formulation of tacrolimus, marketed under the trade name Astragraf XL® was approved by the FDA in 2013. No studies have yet been performed in lung transplant recipients; however they may be available in the future. Despite multiple studies indicating post-dose levels to more accurately predict AUC, most centers utilize trough concentrations for therapeutic drug monitoring (31,32). Target ranges vary according to center-specific protocols and practices, and take into account patient characteristics, such as time post-transplant and rejection and infection history. Generally, target trough concentrations range from 5-15 ng/mL. Tacrolimus displays similar adverse effects to cyclosporine, with perhaps less hypertension and hypercholesterolemia, but more neurotoxicity and diabetes (33-39). Thrombotic thrombocytopenia purpura and hemolytic uremic syndrome have been reported with both cyclosporine and tacrolimus (40). Both cyclosporine and tacrolimus undergo metabolism via the hepatic cytochrome (CYP) P450 3A4 and 3A5 enzymes and p-glycoprotein efflux pumps present on intestinal mucosa,leading to significant drug interactions with CYP inducers (e.g., rifampin, phenobarbital, carbamazepine, phenytoin) and inhibitors (e.g., azoles, macrolides, calcium channel blockers). Additional drug interactions exist for cyclosporine, as it is not only a substrate of CYP 3A4 but also a moderate inhibitor (statins).

Selected data comparing cyclosporine and tacrolimus are shown in *Table 2*. Majority of the trials are small, prospective, randomized studies showing no statistical differences in acute rejection or survival between those treated with cyclosporine or tacrolimus, whether receiving no induction or ATG, AZA or mycophenolate. The most recent study published in 2012 by Treede *et al.* is the largest study to date and showed no difference between cyclosporine and tacrolimus in acute rejection or survival at 3-year, however there was a higher incidence of BOS stage 1 or greater with cyclosporine and it was also shown to be a risk factor for the development of BOS by univariate analysis (46). According to the most recent ISHLT Registry report, tacrolimus was the most frequently used calcineurin inhibitor, 83% at one year post-transplant, 77% at 5 years post-transplant (2).

Anti-proliferative agents

AZA was the first anti-proliferative agent available for use. AZA is converted to 6-mercaptopurine (6-MP) in vivo which then is converted into several compounds that get incorporated into the DNA of replicating cells and halt proliferation (47). AZA is associated with significant leukopenia, thrombocytopenia, anemia, hepatotoxicity (transaminitis and cholestasis), and rarely pancreatitis. Caution must be used when using AZA with xanthine oxidase (XO) inhibitors (e.g., allopurinol). XO is thought to be responsible for converting 6-MP to metabolites. The combination results in significant bone marrow suppression

Table 2 Maintenance immunosuppression

Citation	Immunosuppressant	N	Methods	Outcomes
Griffith *et al.* 1994 (41)	FK506 *vs.* CsA	74	Prospective, randomized	AR: 1.2 *vs.* 2 episodes per 100 patient days, P<0.05 Survival, 1-yr: no difference Bacterial infection: 0.6 *vs.* 1.5 episodes per 100 patient days, P= NS
Treede *et al.* 2001 (42)	Tac *vs.* CsA	50	Prospective, randomized	Freedom from AR, 1 yr: 50% *vs.* 33.3%, P= NS Treated episodes of AR/100 patient days: 0.225 *vs.* 0.426, P<0.05 Survival, 1 yr: 73.1% *vs.* 79.2%, P= NS No difference in infection
Zuckerman *et al.* 2003 (43)	Tac *vs.* CsA	74	Prospective, randomized	Freedom from AR, 1-yr: 46% *vs.* 35%, P=0.774 Treated episodes of AR/100 patient days: 0.22 *vs.* 0.32, P=0.097 Survival, 1-yr: 71% *vs.* 82%, P=0.748 Infections: 0.55 *vs.* 0.7, P=0.059
Hachem *et al.* 2007 (44)	Tac *vs.* CsA	90	Prospective RCT	Composite (Cumulative ≥ A3 AR, ≥ B4 LB, BOS 0-p): 50% *vs.* 84.8%, P=0.002 AR or LB: 41% *vs.* 63%, P=0.036 Freedom from BOS 0-p: Tac > CsA, P=0.1
Neurohr *et al.* 2009 (45)	Tac + MMF	155	Retrospective	Freedom from AR, 1-yr: 74.6% Freedom from AR, 5-yr: 59.5% Freedom from BOS, 1-yr: 95.6% Freedom from BOS, 5-yr: 69.5% Survival, 1-yr: 86.4% Survival, 5- yr: 60.3%
Treede *et al.* 2012 (46)	Tac *vs.*CsA	249	Prospective, randomized	AR, 3-yr: 67.4% *vs.* 74.9%, P=0.118 BOS ≥ stage 1-, 3-yr: 11.6% *vs.* 21.3%, P=0.037 Survival, 1-yr: 84.6% *vs.* 88.6% (NS) Survival, 3-yr: 78.7% *vs.* 82.8% (NS) No difference in infection

FK506, tacrolimus; CsA, cyclosporine;AR, acute rejection; NS, not statistically significant; Tac, tacrolimus; RCT, randomized controlled trial; LB, lymphocytic bronchiolitis; BOS, bronchiolitis obliterans syndrome; MMF, mycophenolate mofetil; yr: year.

and a 75% dose reduction of AZA in combination with XO inhibitors is generally recommended. The typical starting dose is 2 mg/kg IV or orally daily.

Mycophenolate is the most frequently used antiproliferative agent used according to the most recent ISHLT Registry report (2). Mycophenolate mofetil and mycophenolate sodium are converted to the active metabolite, mycophenolic acid (MPA), which inhibits inosine monophosphate dehydrogenase (IMPDH), the enzyme responsible for T and B lymphocyte production. Inhibiting this enzyme results in decreased T and B lymphocyte proliferation. Because lymphocytes lack the ability to utilize salvage pathways for nucleotide synthesis and thus rely on the IMPDH pathway, mycophenolate is selective for T and B lymphocyte proliferation inhibition (47). Mycophenolate undergoes rapid absorption and conversion to MPA. MPA is metabolized hepatically into mycophenolic acid glucuronide (MPAG). MPAG is excreted via bile into the intestines, where it is converted back to the active metabolite, MPA, resulting in a second peak concentration in the plasma. Doses range from 1-1.5 g IV or oral twice daily. Therapeutic drug monitoring is available for mycophenolate, with AUC being the optimal parameter for measuring treatment response. Trough values have

shown poor predictive response (48-50). LSS calculations for estimation of AUC in lung transplant patients are also availablehowever therapeutic drug monitoring has not been firmly established (51). Principle adverse effects of mycophenolate are leukopenia, thrombocytopenia, and gastrointestinal disturbances (diarrhea, abdominal pain, nausea, vomiting). Initial use of mycophenolate involved rescue therapy following development of BOS, with stabilization of pulmonary function testing after switching from AZA (52). In a prospective, randomized trial of 81 lung transplant recipients comparing azathiopine to mycophenolate in combination with cyclosporine and CS, there were no differences in biopsy-proven or clinical rejection, survival, infection, or adverse drug events at 6-month (53). A subsequent prospective, randomized multicenter study comprising 315 lung transplant recipients also showed no difference between AZA and mycophenolate when used in combination with cyclosporine and CS in the outcomes of acute rejection, BOS, and survival at 3-year, however a greater percentage of patients discontinued AZA than mycophenolate (59.6% *vs.* 46.5%) (54).

Srl and evl are two newer antiproliferatives in the mTOR inhibitor class. Both bind to intracellular immunophilin FK506 binding protein like tacrolimus, however unlike tacrolimus the complexes they form do not inhibit calcineurin but instead bind to mTOR, which is a signaling pathway needed to promote progression of the cell cycle from G1 to S phase. The end effect of mTOR inhibitors is a decrease in T lymphocyte activation and proliferation (47). Srl is available as oral tablets and an oral solution. Doses range from 0.5-6 mg daily, with target trough values ranging 5-15 ng/mL. Evl is available as oral tablets. Doses range from 0.25-3 mg twice daily, with target trough values ranging 5-15 ng/mL. Notable adverse effects include decreased wound healing, leukopenia, thrombocytopenia, hypertriglyceridemia, proteinuria, and pneumonitis. Both are metabolized by CYP 3A4 and therefore have similar drug interactions as tacrolimus. The role of mTOR inhibitors in lung transplant is still being identified. They may be used in conjunction with or substituted for either calcineurin inhibitors or other antiproliferative agents. The most common reasons for use include kidney dysfunction due to calcineurin inhibitors, onset of BOS, and malignancy (55-57). For those who exhibit kidney dysfunction, adding an mTOR inhibitor and reducing the calcineurin inhibitor dose has been shown to improve kidney function (55,58,59).Additionally, due to their antiproliferative and anti-fibroblast effects (60), mTOR inhibitors have been used in lung transplant recipients with BOS to help slow progression. Indeed small, retrospective studies have shown stabilization or improvement in pulmonary function testing in lung transplant recipients with BOS (55,56,61,62). Two studies used srl immediately post-transplant and reported significant wound dehiscence and airway complications, leading to death in some patients (63,64), so mTOR inhibitors should not be used until the anastomosis and airways have healed. In 2006, Snell and colleagues performed a prospective randomized controlled trial comparing AZA and 3th month conversion to evl in 213 lung transplant recipients also maintained on cyclosporine and CS. The composite endpoint of efficacy failure (>15% FEV_1 decline from baseline, graft loss, death or loss to follow up) occurred in 33.9% *vs.* 21.8% of patients at 12-month (P=0.046), however there was no difference in this composite endpoint at 24-month. The authors concluded that evl did demonstrate a slowing in loss of pulmonary function over time (65). Most recently, Sacher and colleagues published data on 24 lung transplant recipients who were converted to srl prophylactically *vs.* AZA/MMF, one year post-transplant. Of the 19 patients who remained on long-term srl, a trend toward a reduction in the incidence of BOS and improved survival was reported (66). Larger, randomized controlled trials are needed to more fully elucidate the effect of mTOR inhibitors in the prevention of BOS.

Corticosteroids (CS)

CS have been used in solid organ transplant since the very beginning and have not only remained a corner stone of both induction and maintenance immunosuppression but they are also used to treat acute cellular rejection (ACR) as well. The most commonly used CS in solid organ transplant are methylprednisolone and prednisone. CS are known to have antiinflammatory properties and exert their effects in a variety of ways, including inhibiting the NFkB pathway, preventing T cell proliferation, decreasing macrophage activation, inhibitingcytokine production and altering lymphocyte migration (67). According to the most recent ISHLT registry report, CS continue to be used by almost all transplant centers, at one and five years post-transplant. Initial doses range from 500-1,000 mg given intraoperatively, and are gradually tapered over weeks to months to 5-10 mg per day for maintenance. Short and long term use of CS is associated with significantside effects, including hypertension, weight gain, hyperlipidemia, hyperglycemia and diabetes mellitus, osteoporosis and increased risk of fractures, increased risk of cataracts, poor

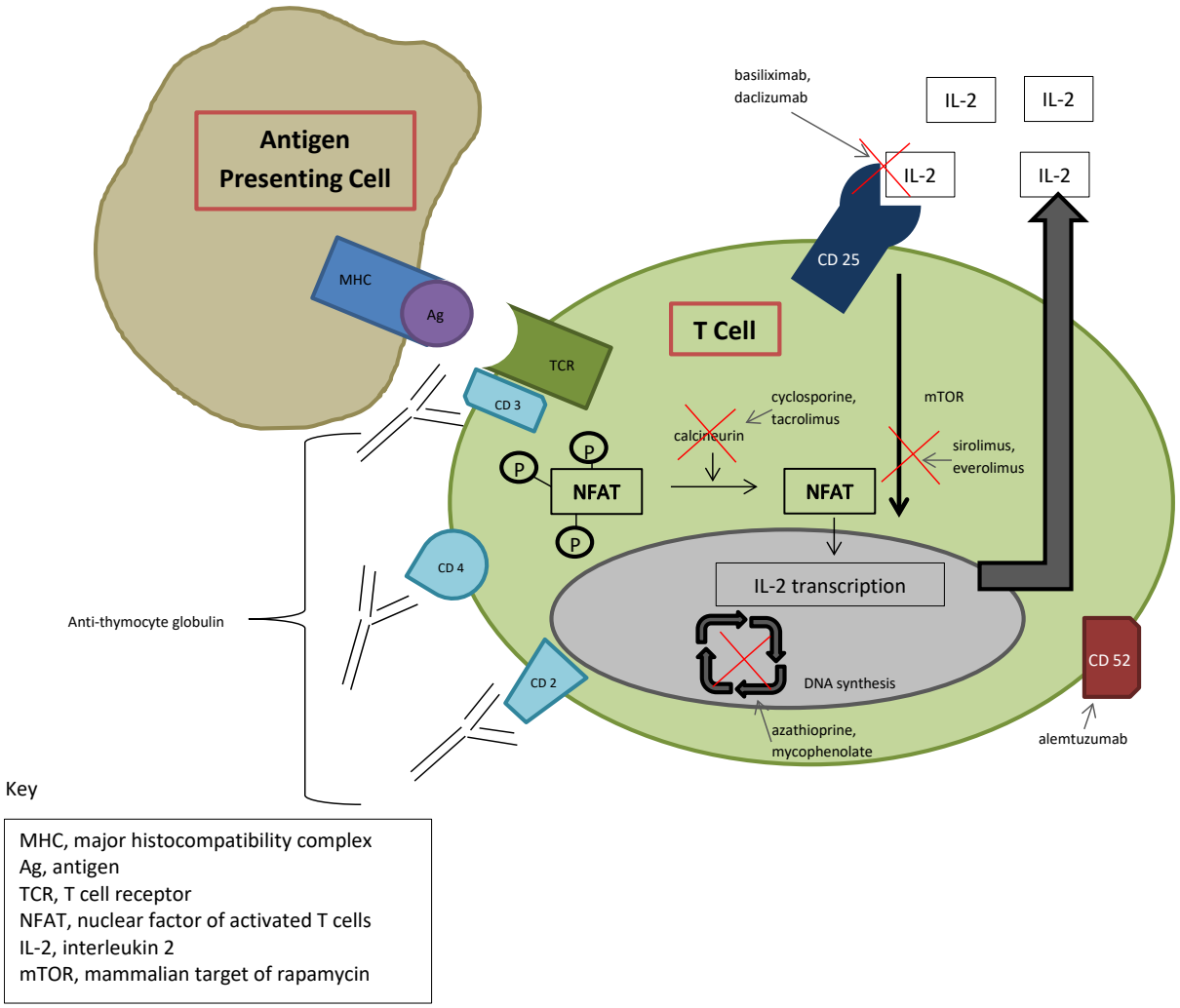

Figure 1 Mechanisms of action of immunosuppressive agents.

wound healing, psychiatric disturbances and infectious complications.Data on steroid-free regimens in lung transplantation is lacking and at best shows limited success (68,69). Complete steroid-withdrawal should be avoided at the present time, owing to a significant risk of allograft dysfunction; however, doses should be lowered as quickly and as safely as possible, and maintainthe lowest possible doses with the goal of stable and optimal lung function while avoiding and minimizing drug-related adverse effects (*Figure 1*).

Antihumoral therapy

Generally immunosuppression is employed to suppress cell mediated immunity by targeting T cell function and proliferation as rejection is usually a cell mediated phenomenon. However the role of humoral or antibody-mediated rejection (AMR) in solid organ transplant recipients has become more evident over the years. Antibody mediated rejection has been identified and characterized in other organs but remains poorly defined in lung transplant recipients. No agreed upon pathologic criteria exists to date in lung transplantation (70,71). Mechanisms by which anti bodies, which usually are donor specific antibodies (DSA), produce injury are not yet well described. Injury may be complement mediated or complement independent (72). No universally agreed upon management strategy exists for these antibodies. Use of intra venous immunoglobulin (IVIG), one of most commonly used treatments with a relatively low side effect

profile, with or without plasmapheresis, peritransplant and after development of DSA post-transplant resulted in improvement in certain parameters such as acute rejection and BOS at a single institution (73). In a study reported by Hachem and colleagues, use of IVIG combined with rituximab, a monoclonal anti CD20 antibody, *vs.* IVIG to clear newly acquired DSA showed improved survival and freedom from BOS in patients who cleared DSA after treatment. However there was no improvement in clearance of DSA with addition of rituximab to IVIG (74). Plasmapheresis is mainly used for antibody removal from circulation in suspected cases of humoral rejection which do not respond to steroids, leading to clinical improvement (75). Bortezomib, an inhibitor of 26S proteasome that leads to plasma cell apoptosis, has been used successfully in case reports to treat possible acute humoral rejection in lung transplant recipients (76,77). Hyperacute rejection due to pre formed antibodies against donor HLA antigens has become uncommon due to ongoing cross match screening. Treatment with IVIG, plasmaphresis, rituximab, antithymocyte globulin and eculizumab has been described in various case reports with variable degree of success (78-80).

Novel approaches

Aerosolized calcineurin inhibitors

A number of reports have been published regarding the use of aerosolized cyclosporine. In 1996, Iacono and colleagues published a report of histologic improvement of obliterative bronchiolitis (OB) and stabilization of pulmonary function testing in 7 lung transplant recipients who received aerosolized cyclosporine as rescue therapy (81). Shortly thereafter, the use of aerosolized cyclosporine to treat refractory acute rejection in 9 lung transplant recipients was associated with histologic improvement in 8 of 9 subjects, improvement in pulmonary function testing, a reduction in cycles of pulse dose CS and ATG, reduction in oral prednisone dose, and reduction in episodes of pneumonia was also observed, compared to 22 historical controls (82). Both reports showed no additional renal or hepatic toxicity with the use of aerosolized cyclosporine. A larger case-control study was subsequently undertaken and demonstrated a survival advantage in lung transplant recipients with biopsy-documented OB compared to conventional immunosuppression (83). While the most well-studied randomized placebo-controlled trial of aerosolized cyclosporine did not show a reduction in the primary endpoint of rate of ACR, it also demonstrated a survival advantage compared with conventional immunosuppression, and showed an improvement in chronic rejection-free survival (84). Despite these results, an FDA-approved formulation of aerosolized cyclosporine is still currently unavailable. Animal studies aiming to characterize aerosolized tacrolimus pharmacokinetics and safety have been published (85-87). The first case report of using tacrolimus via inhalation in a human lung transplant recipient with BOS was recently published demonstrating improved functional capacity and oxygenation after one week of therapy (88). More data are needed to determine the optimal use of aerosolized calcineurin inhibitors but this therapeutic approach seems promising.

Azithromycin

Azithromycin is a macrolide antibiotic with anti-inflammatory and immunomodulatory effects (89). These effects, in conjunction with the beneficial effects of maintenance azithromycin seen in cystic fibrosis patients led to pilot studies of azithromycin in lung transplant recipients with BOS (90-93). In 5 of 6 patients, thrice-weekly azithromycin for 13 weeks demonstrated an average 17% improvement in FEV_1 (92) and an average 18% improvement in FEV_1 after 12 weeks of therapy in 8 others (93). A retrospective analysis of 20 lung transplant recipients also demonstrated an improvement in FEV_1 after 12 weeks of azithromycin therapy (average 110 mL from baseline) (94). However, not all patients respond to azithromycin therapy (95-97). Evidence suggests airway neutrophilia and elevated interleukin-8 bronchoalveolar (BAL) concentration may be predictors of response (95,97,98). Furthermore, studies have indicated that early initiation of azithromycin, e.g., BOS 0-p, may have more of an impact on preventing disease progression and may improve survival (97,99,100). In a randomized, placebo-controlled trial of 83 lung transplant recipients, there was a significant reduction in the incidence of BOS at 2-year in those who received azithromycin prophylactically compared to those who did not (12.5% *vs.* 44.2%, P=0.0017) (101). There was also a significant difference in BOS-free survival (HR 0.27, P=0.020), although overall survival was similar between groups. Collectively these data suggest early initiation of azithromycin in lung transplant recipients may prevent the incidence of BOS and prolong BOS-free survival, and may improve or stabilize pulmonary function after the onset of BOS, particularly in those with neutrophil- and IL-8-predominant BAL.

Extracorporeal photopheresis (ECP)

ECP was developed initially for treatment of cutaneous T cell lymphomabut has been utilized in variety of disease states including solid organ transplantation. The process involves leukopheresis followed by incubation of the isolated cells with 8-methoxypsoralen (8-MOP) and subsequent activation of 8-MOP with ultraviolet A radiation. These cells are then reinfused into the patient. 8-MOP activation causes DNA cross linkage and apoptosis. Reinfusion of these apoptotic cells generate T regulatory cells (T regs) and increased production of IL-10 and transforming growth factor beta. Exact mechanisms by which these immunomodulatory effects are produced are not well understood. At present, clinical studies assessing efficacy of ECP in lung transplant recipients are limited to retrospective single center studies done in patients showing declining lung function. No trials to assess the prophylactic effect of ECP on development of BOS by starting ECP immediately post-transplant have been done to date. In a study by Morrell and colleagues, 60 lung transplant patients received ECP in addition to conventional immunosuppression for treatment of progressive BOS. Fifteen patients (25%) showed an improvement in FEV_1 and rest showed a reduction in rate of decline in FEV_1 which persisted at 12 months after initiation of ECP (102). Another study done by Jaksch and colleagues, 51 lung transplant recipients who developed BOS and did not respond to augmentation of immunosuppression and azithromycin, received ECP.Thirty-one patients (61%) showed improvement or stabilization of lung function while 20 patients (39%) had continued decline in lung function and did not respond to ECP. Survival rate after start of BOS at 1, 3 and 5 years was significantly better in treatment responsive group (103).These studies did not identify any significant characteristics among lung transplant recipients that could predict the response to ECP. Recently a retrospective single center study done by Greer and colleagues assessed clinical efficacy of ECP treatment in lung transplant recipients with azithromycin-refractory chronic lung allograft dysfunction (CLAD) and attempted to associate clinical response to several CLAD phenotypes. Sixty-five lung transplant recipients were diagnosed and graded for graft dysfunction in accordance with ISHLT BOS criteria and were started on ECP treatment while showing deterioration or no improvement despite taking azithromycin which was started after reversible causes of graft dysfunction were excluded. Thirty-five patients (54%) showed improvement or stabilization of FEV_1 while 30 patients showed >10% decline in FEV_1. Three CLAD phenotypes, restrictive allograft syndrome, defined by TLC ≤90% of baseline, non neutrophilic CLAD, patients demonstrating BAL neutrophilia <15% and rapid decliners, patients suffering a >100 mL/month decline in FEV_1 before ECP initiation showed that they were less likely to benefit from ECP treatment. Significant survival benefit was noted in the ECP responsive group when compared to the ECP refractory group (104). Randomized clinical trials are needed to better evaluate the benefit and possibility of early use of ECP after onset of CLAD in lung transplant recipients.

Statins

Statins, 3-hydroxy-3-methylglutaryl coenzyme Areductase inhibitors, have been shown to have properties which may have a potential beneficial impact on lung allograft function post-transplant. They have been shown to reduce the gamma interferon induced expression of major histocompatibility molecules on cells, increase the number of $CD4^+CD25^+$ T regs, inhibit growth factor expression in lung fibroblasts and inhibit the development of obliterative airway disease in animal models (105-108).

These abovementioned immunomodulatory and anti-fibroproliferative properties have potential benefit for lung transplant recipients. However, clinical evidence in lung transplant recipients is limited to retrospective single center studies only. Johnson and colleagues showed improved 6-year survival in statin group compared to controls, 91% *vs.* 54%, as well as reduced rates of acute rejection and BOS (109). Li and colleagues showed improved survival and maintenance of lung function associated with post-transplant use of simvastatin in a single center cohort analysis of 502 lung transplant recipients (110). Prospective randomized trials are needed to confirm these findings, compare different statins and determine the optimal dose.

Pirfenidone

Pirfenidone is an anti-fibrotic agent used to treat pulmonary fibrosis. It inhibits growth-factor dependent proliferation of fibroblasts, T cell proliferation and activation, and may inhibit dendritic cell activation and function (111-115), and may be a potential therapeutic strategy for the treatment of CLAD. Thus far two case reports of pirfenidone use in human lung transplant have been published (116,117). The first reported a mild increase in FEV_1 following progressive

Table 3 Summary of stages and types of therapy

Induction immunosuppressants (Goal: prevent acute cellular and antibody-mediated rejection; delay initiation of nephrotoxic immunosuppressants)
Interleukin 2 receptor antagonists (non-depleting monoclonal antibody)
Daclizumab (Zenapax©)
Basiliximab (Simulect©)
Anti-thymocyte globulin (cell depleting polyclonal antibody preparation)
Equine (ATGAM©)
Rabbit (Thymoglobulin©)
Anti-CD 52 monoclonal antibody (cell-depleting)
Alemtuzumab (Campath©)
Maintenance immunosuppressants (Goal: prevent acute cellular antibody-mediated rejection; prevent chronic lung allograft dysfunction)
Calcineurin inhibitors
Cyclosporine (Sandimmune©, Neoral©)
Tacrolimus (Prograf©)
Anti-proliferative agents
Azathioprine (Imuran©)
Mycophenolatemofetil (CellCept©)
mTOR inhibitors
Sirolimus (Rapamune©)
Everolimus (Zortress©)
Corticosteroids
Methylprednisolone (Solu-Medrol©, Medrol©)
Prednisone (Deltasone©)
Acute cellular rejection, treatment
Methylprednisolone (Solu-Medrol©, Medrol©)
Anti-thymocyte globulin (Thymoglobulin©)
Alemtuzumab (Campath©)
Antibody-mediated rejection, treatment
Plasmapheresis
IVIG
Rituximab (Rituxan©)
Bortezomib (Velcade©)
Chronic lung allograft dysfunction, treatment
Azithromycin (Zithromax©)
Extracorporeal photopheresis
Statins
Pirfenidone

IVIG, intra venous immunoglobulin.

decline with no evidence of infection or rejection and failure to respond to azithromycin, montelukast and fundoplication (116). The second reported a slower rate of decline in forced vital capacity, FEV_1, and a mild increase in total lung capacity in a lung transplant recipient with restrictive allograft syndrome (117). Given these findings, further study of pirfenidone in human lung transplantation is warranted.

Treatment

ACR, AMR and CLAD are discussed in-depth elsewhere. Specific treatment protocols vary from center to center, but options are limited to high-dose or "pulse" CS (e.g., methylprednisolone 10-15 mg/kg IV daily × 3-5 days), particularly for initial treatment or minimal-mild grade ACR; ATG (1.5 mg/kg IV daily × 3-5 days) or alemtuzumab (30 mg IV once) for moderate-severe grade ACR or steroid-resistant/steroid-refractory ACR. Therapies available for treatment of AMR include plasmaphereis (5-6 cycles), IVIG (1-2 g/kg over 3-6 days), rituximab (375 mg/m^2 IV weekly × 4 doses or 1,000 mg IV every 2 weeks × 2 doses), and/or bortezomib (1-1.3 mg/m^2 every 72 hours × 4 doses). Treatment options for CLAD are even more limited, and there is currently no agent available to date that reverses that process and restores lung function, other than re-transplant when available. Therapies targeting the processes of CLAD either prevent the onset of CLAD, or prevent and delay its progression. These include azithromycin, ECP, the statins, and pirfenidone. Augmentation of immunosuppression with ATG, alemtuzumab, addition or substitution of an mTOR inhibitor to the maintenance regimen, substitution of mycophenolate for AZA or of tacrolimus for cyclosporine, are additional strategies that have been employed with varying success (*Table 3*).

Summary

Our understanding of the underlying mechanisms and clinical presentation of acute allograft rejection and CLAD continue to evolve. Immunosuppressive regimens have significantly contributed to the improvement of the survival of lung transplant recipients. Despite the progress in the management of lung transplant recipients, they continue to be at high risk of treatment-related complications, poor allograft and patient survival. Randomized clinical trials are needed to allow the development of better agents, regimens and techniques to address above mentioned issues

and reduce morbidity and mortality among lung transplant recipients.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Scheffert JL, Raza K. Immunosuppression in lung transplantation. J Thorac Dis 2014;6(8):1039-1053. doi: 10.3978/j.issn.2072-1439.2014.04.23

Five-year update on the mouse model of orthotopic lung transplantation: Scientific uses, tricks of the trade, and tips for success

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Abstract: It has been 5 years since our team reported the first successful model of orthotopic single lung transplantation in the mouse (1). There has been great demand for this technique due to the obvious experimental advantages the mouse offers over other large and small animal models of lung transplantation. These include the availability of mouse-specific reagents as well as knockout and transgenic technology. Our laboratory has utilized this mouse model to study both immunological and non-immunological mechanisms of lung transplant physiology while others have focused on models of chronic rejection (1-19). It is surprising that despite our initial publication in 2007 only few other laboratories have published data using this model (20-25). This is likely due to the technical complexity of the surgical technique and perioperative complications, which can limit recipient survival (9,26). As two of the authors (XL and WL) have a combined experience of over 2500 left and right single lung transplants, this review will summarize their experience and delineate tips and tricks necessary for successful transplantation. We will also describe technical advances made since the original description of the model (1,5,6).

Keywords: Mouse model; orthotopic lung transplantation; surgical complications; evaluation

Submitted May 07, 2012. Accepted for publication Jun 05, 2012.
doi: 10.3978/j.issn.2072-1439.2012.06.02
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2012.06.02

Introduction

As of 2011 our laboratory has successfully completed over 4,500 mouse orthotopic lung transplants as a part of investigator-initiated studies and collaborations with biotechnological companies (1-7,27-30). While in the beginning of our experience technical obstacles were encountered, after an initial learning curve complications are now rare (less than 1%, not including post-transplantation atelectasis). Other laboratories have reported technical success rates of around 80-90% (9,26). As we have already described the rudimentary steps of this procedure (1,5) , here we will focus only on major surgical complications that hinder experimental success. We will also discuss the causes of technical failures and steps that can be taken to prevent them.

Complications

Pneumothorax

The first type of pneumothorax (Type I pulmonary injury) stems from technical errors that occur due to excessive handling of the lung. This results in a severe air leak, mostly because of a bronchial tear, or damage to the lung surface. This becomes evident immediately after graft reperfusion and reaeration. This type of injury is worth repairing if the area of injury is small or the recipient is a

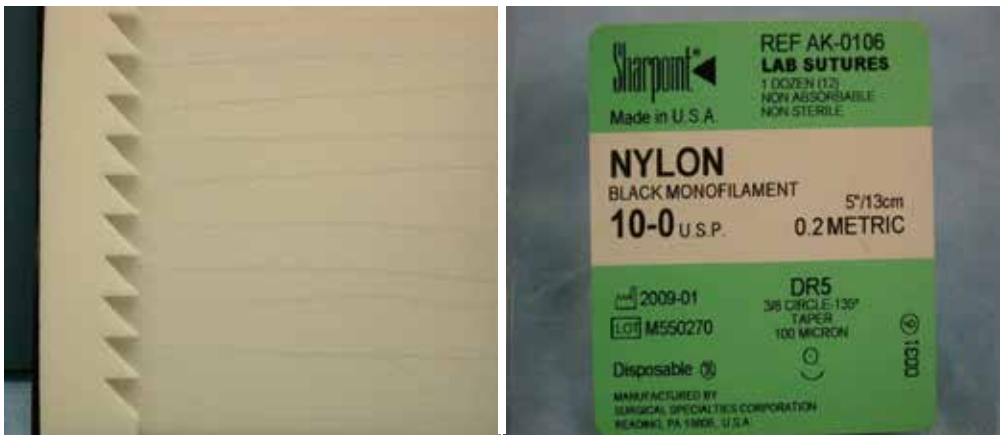

Figure 1 10-0 Nylon for suturing tiny hole of bronchus.

Figure 2 Animal Surgical Glue for sealing small air leak of graft lung.

very rare transgenic mouse. Although the mouse bronchus and lung tissue are very friable, one can still repair this form of damage by placing a shorter cuff on the torn bronchus to incorporate the area of damage into the cuff or utilize 10-0 nylon suture (Sharpoint AK-D106, Surgical Specialties Corporation, *Figure 1*) to repair a small hole in the donor or recipient bronchus. As for tiny defects on the graft surface one can use tissue glue to seal it (Webglue, Webster Veterinary Supply Inc, *Figure 2*). In our experience a successful repair of this type of injury is easy to achieve and the graft can often be salvaged.

The second type of pneumothorax (Type II pulmonary injury) is more dangerous because the lung injury is small and may not be evident immediately upon release of the hilar structures. This is usually discovered only after extubation of the animal and is often manifested by respiratory distress and difficulty in initiating spontaneous respirations. Without immediate reintubation the recipient will die within 1 or 2 minutes and is unlikely to be resuscitated. In order to salvage this situation the animal must be quickly reintubated and the upper part of the abdomen opened. This will allow immediate diagnosis of the problem as either the left or the right side of the diaphragm will bulge down with an obvious tension pneumothorax. In our experience a Type II pulmonary injury can occur either on the side of the transplant or native lung. While the reason for this injury can be difficult to determine it may be related to the process of mechanical ventilation. In the mouse the respiratory rate varies between 60-230 breaths per minute (bpm). The tidal volume can vary between 0.09-0.38 mL/breath resulting in a minute ventilation of 11-36 mL/min (31). Despite this physiology our preferred mouse ventilator settings include a tidal volume of 0.5 mL and a respiratory rate of 110-120 bpm, which yield minute ventilations exceeding the normal range. Other laboratories performing similar procedures recommend even higher tidal volumes (26). In our experience decreasing the minute ventilation for extended portions of the procedure leads to poor long-term outcome (unpublished data). During single lung ventilation, however, this high volume positive-pressure may cause barotrauma and volutrauma, which may lead to a Type II pulmonary injury (32). While serious, this complication is very rare (from our database we have encountered fewer than 20 cases in 5 years), and the severity of pulmonary damage is unpredictable. In order to prevent this complication we: (I) reduce the tidal volume from 0.5 mL to 0.35-0.40 mL when single lung ventilation is initiated and maintain this low tidal volume until the lung graft is implanted and (II) inflate the lung graft for no more than 1-2 seconds after implantation. However, if a pneumothorax does occur the only possible treatment

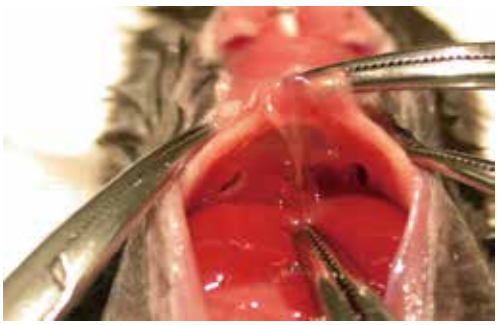

Figure 3 Diaphragm window for treatment of type II pulmonary injury.

is to reintubate the animal immediately, incise the upper abdomen and cut a small window in the diaphragm to release the air that has collected under tension in the chest. After recipient hemodynamics have stabilized the diaphragmatic window is left open and the abdominal incision is closed (*Figure 3*). If needed, one can close the diaphragm window at a later time point. However, it is unlikely that these salvage maneuvers will result in a ventilated lung at the time of sacrifice as atelectasis will occur after this type of injury. Therefore, mice can generally not be used for data generation if this complication arises.

Graft atelectasis

The anatomy of the mouse lung differs from that of other species (33). Total lung capacity (TLC) of the mouse is about 1ml compared to 10 ml in the rat. The alveoli of the mouse lung are significantly smaller than those of the rat (34) and the airways constitute a large percentage of the lung volume in the mouse (11%) compared to the rat (5.7%). Cartilage is present in the mouse trachea, but is less well organized than in other species. Mouse lungs also have fewer respiratory bronchioles and airway generations than humans. Two other significant features of the mouse lung anatomy are the thinness of the respiratory epithelium and the relatively large airway lumen (35). This large airway caliber may reduce the flow-resistive load in this small animal with a rapid respiratory rate (33). All of these characteristics work in favor of preventing atelectasis of the mouse lung in comparison to other animal models. Nevertheless, it has been brought to our attention that non-alloimmune-mediated collapse of the grafted lung, which is not associated with graft rejection, but rather due to technical problems, is the most common complication encountered by other laboratories. Thus, in our opinion, pulmonary atelectasis after transplantation is generally due to methodologic problems rather than inherent anatomic considerations.

Based on our experience and serial sacrifice, pulmonary collapse that is unrelated to graft rejection usually occurs on the third to fifth post-operative day after transplantation and is often not diagnosed until the time of sacrifice. The diagnosis of atalectasis is further complicated by the fact that mice do not manifest any outward signs of shortness of breath when this occurs (6). Furthermore, while non-invasive diagnostic tools such as small animal MRI are available to monitor pulmonary graft function after transplantation. the routine use of such modalities is impractical (*Figure 4*) (29). Thus, we contend that the best method for preventing graft atelectasis is to pay meticulous attention to surgical details and rely on several "tricks" that we have accumulated over the years in order to prevent this complication.

In our mouse model, we believe pulmonary collapse occurs due to two main reasons. The first one involves pathophysiologic changes in pulmonary parenchyma after transplantation and the second one is technical failure. Graft ischemia-reperfusion injury alters graft compliance, increases air flow resistance and contributes to atelectatic collapse of the lung (36). Anastomotic problems of the small mouse bronchus also can lead to atelectasis and anastomotic ischemia followed by remodeling and granulation tissue formation, which leads to bronchial wall thickening and significant airway obstruction (37-39). Technical problems due to poor orientation of the cuff, donor to recipient cuff mismatch and other mishaps can lead to atelectatic collapse of the lung graft as well. While some have demonstrated that cuffing the bronchial anastomosis can be problematic and may lead to airway stenosis in the rat (36) the small size of the bronchial structures in the mouse makes suturing this structure nearly impossible (unpublished observations). Thus, while retaining the cuff technique for the bronchial anastomosis we have gradually modified the surgical approach and have made significant revisions in the method for cuffing this structure. First, we now shorten the donor bronchus as close as possible to the secondary carina in order to reduce anastomotic ischemia and improve the bronchial blood supply (40-43). This type of maneuver also results in the need to shorten the venous cuff in order to prevent rotation and torsion of the hilar structures. Second, we use as big a cuff as possible in order to reduce air flow resistance (with gain of experience some microsurgeons

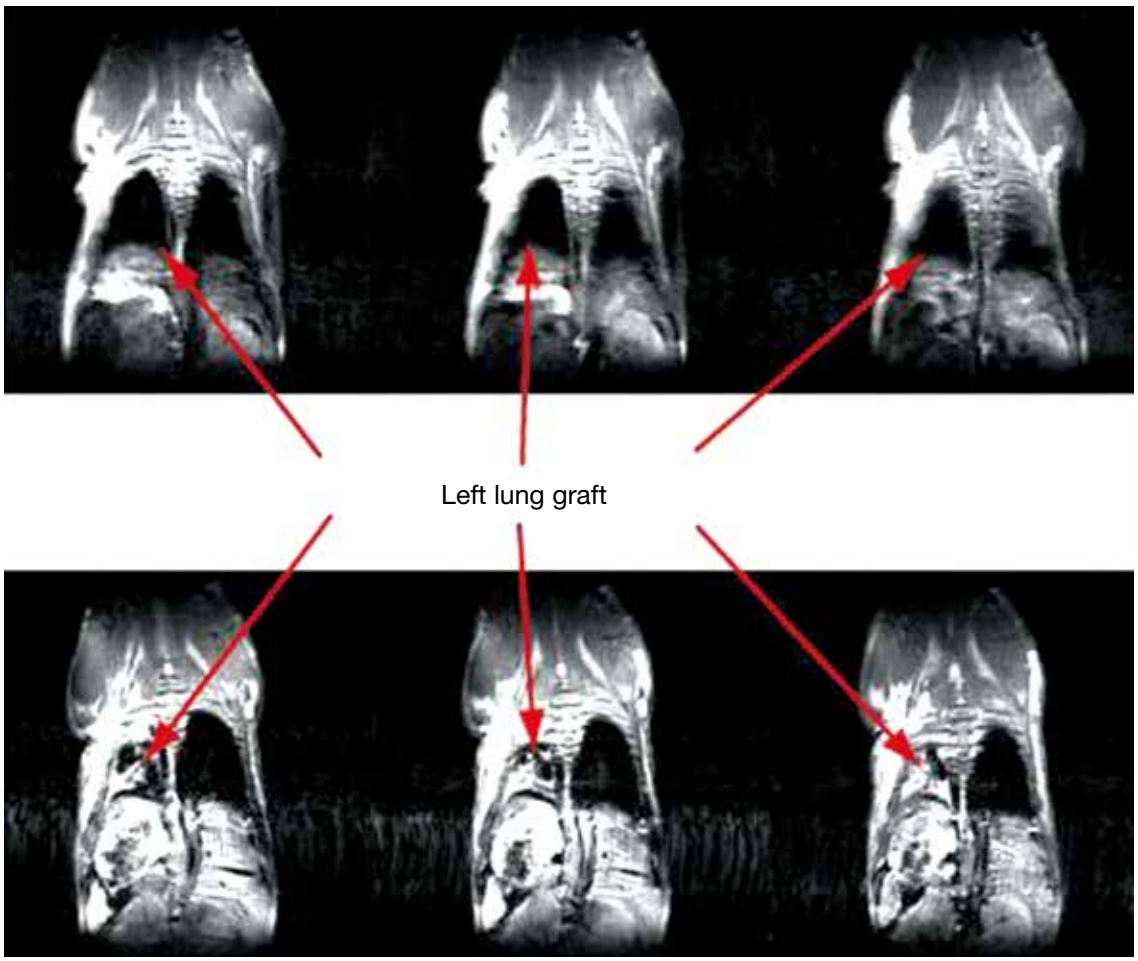

Figure 4 MRI images obtained 3 days after lung transplantation reveal ventilation of lung graft on three serial coronal images (top) and graft atelectasis in a different animal (bottom). As previously described (29) aerated lung looks black on MRI due to lack of signal intensity while a consolidated lung looks white due to the high intensity of this water-rich structure when atelectatic.

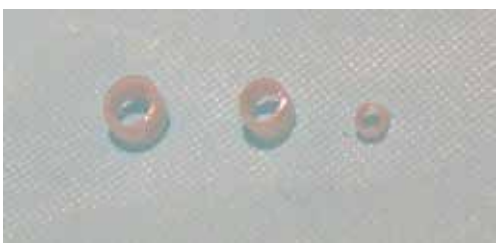

Figure 5 Adjustment of bronchus cuff size. From L to R, the size is 18#, 20# and 24#, which are used for bronchus, pulmonary vein, and pulmonary artery, respectively.

now cuff most strains of mice with a cuff made from a #18 angiocatheter instead of a #20 as we had previously described (5) (*Figure 5*). As the hilar anatomy of each strain differs (C3H and CBA/Ca mice for example have comparably foreshortened hilar structures) the size of the cuff must match each bronchus in order to achieve not only optimal ventilation, but also prevent venous complications (see below). After these modifications our incidence of graft atelectasis has decreased to <5%.

Pulmonary venous thrombosis

This complication does not usually lead to immediate post-operative demise and is usually discovered at autopsy either due to recipient death before the scheduled sacrifice or at the time of sacrifice. Gross appearance of the graft can be diagnostic of pulmonary venous thrombosis as the lung is dark red or black, is grossly enlarged and firm. Often a

Table 1 Classification and grading of pulmonary allograft rejection

A: Acute rejection	with/without	B: Airway inflammation-lymphocytic bronchitis/bronchiolitis
Grade 0: None		Grade X: Ungradeable
Grade 1: Minimal		Grade 0: None
Grade 2: Mild		Grade 1R Low grade
Grade 3: Moderate		Grade 2R: High grade
Grade 4: Severe		
C: Chronic airway rejection: bronchiolitis obliterans		
0: Absent		
1: Present		
D: Chronic vascular rejection: accelerated graft vascular sclerosis.		

"R" denotes revised grade to avoid confusion with 1996 scheme. 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.

clot can be seen in the pulmonary vein. At times the right upper lobe of the native lung can be involved as well due to propagation of clot. Venous thrombosis is usually caused by pulmonary vein torsion or compression of the venous outflow from a large bronchus cuff. Thus, one must balance the "trade off" of a large bronchial cuff, which results in a lower incidence of graft atelectasis, but a higher incidence of pulmonary venous thrombosis. Nevertheless, the incidence of this complication can be reduced to very low levels by meticulous attention to detail in preventing venous torsion and proper cuffing of the hilar structures to reduce rotation of the lung by an imbalance between the bronchial and venous cuffs.

Evaluation

Evaluation of technical success and rejection

After technically successful lung transplantation the graft should be well aerated and perfused, even in the face of mild acute rejection. As mentioned above, unlike cardiac allografts, where early technical failure can be manifested by cessation of heart beat, the evaluation of the technical success of lung transplantation requires either gross inspection after sacrifice or radiographic imaging. Conventional imaging modalities, such as X-rays, lung perfusion scintigraphy or angiography may be useful in humans and large animals, but are not practical for large throughput experiments in mice (44-48). Either the resolution of these techniques is not suitable to evaluate subtle pathological changes, or the techniques cannot be performed on a serial basis in the same animal (36). Greschus suggested Flat-Panel Volumetric Computer Tomography (fpVCT) as a precise tool to assess the success of rat orthotopic lung transplantation that can be used to follow the process of graft rejection with very high spatial resolution (36). Such a technique, however, might not be applicable to the mouse due to its small size. We have recently focused on small animal MRI to evaluate pulmonary pathology and have found this technique to be a highly reproducible non-invasive approach to visualize anatomic pathology of the lung such as pulmonary collapse due to either advanced acute rejection or atelectasis (29) (*Figure 4*). However, we realize that this modality might not be easily accessible to all laboratories and thus to date there still is no easily accessible, effective and precise imaging modality to evaluate the grafted lung for technical success or rejection. Our current practice thus relies on grading rejection based on strict histologic criteria, which mirror human graft evaluation. In 1990, the International Society for Heart and Lung Transplantation (ISHLT) adopted a "Working Formulation of the Standardization of the Nomenclature in the Diagnosis of Lung Rejection," which was revised in 1996 and again in 2007 (49). We have thus adapted this grading scale for the mouse model (*Table 1*). The development of better imaging modalities in the future may facilitate serial monitoring of graft outcome and early detection of technical failures.

Evaluation of graft function

Mouse orthotopic lung transplantation is a very useful model not only because it mimics human lung transplantation, but because the investigator can collect

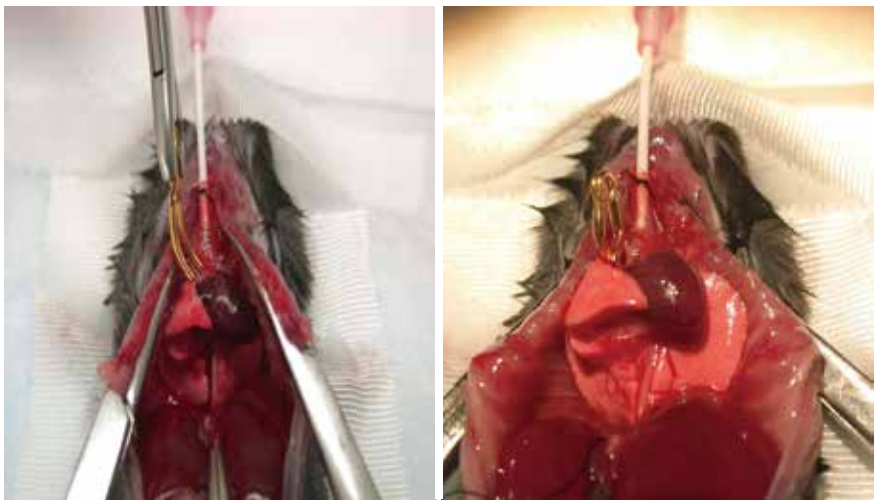

Figure 6 Occlude the hilum of native non-transplanted lung with clip.

Figure 7 Occlude the hilum of native non-transplanted lung with 6-0 silk.

samples that mimic clinical investigations of human lung transplant recipients, such as arterial blood gases (ABGs), bronchioalveolar lavages (BALs), and lung parenchymal tissue. The most important tests, such as spirometry and plethysmography have been reported by some groups (21,50), but are not currently routinely used by us. To date our laboratory has relied mostly on arterial blood gas (ABG) measurement of a mouse supported solely by the lung graft after hilar clamping of the native lung to evaluate function as well as bronchoalveolar lavage, wet dry ratio, histology, FACS, and immunohistochemistry. We have focused on these tests as they mirror the techniques of periopertaive graft assessment used in the clinics. Some of these techniques are described below.

Arterial Blood Gas measurement (ABG)

Our laboratory has relied on ABG measurement to assess graft function (1,6,16,18,19).

(I) Anesthetize the recipient mouse with an intraperitoneal (i.p) injection of Ketamine (5-6 μg/g) and Xylazine (7-8 μg/g). This is about 2/3 of the regular dose used during the actual transplantation procedure as often after transplantation the recipient cannot tolerate a regular dose of anesthetics (5).

(II) Initiate mechanical ventilation of both lungs with 100% FiO_2 for 4 minutes prior to hilar clamping.

(III) Occlude the hilum of the native non-transplanted lung (use either a clip or a 6-0 silk tie. *Figures 6*, 7) and ventilate 4 to 10 more minutes prior to drawing blood.

Here, the microsurgeon needs to detach the right lower lobe from the esophagus very carefully and free the entire right lung (in case of left lung transplantation) from connective tissue without bleeding. The total circulating blood volume in the mouse is only around 2 mL (6-8% of whole body weight), and literally any blood loss will affect hemodynamics, result in circulatory instability and impact the ABG measurement. Ventilate both lungs for 4 minutes before occluding the native right lung in order to assess the function of the graft. The duration of single lung ventilation prior to drawing ABG is controversial and must be tailored based on experimental conditions (1,26). After transplantation either ischemia-reperfusion injury or graft rejection

Figure 8 The current method for BAL.

can cause severe lung graft dysfunction. Poor oxygenation as a result of this type of injury can lead to myocardial ischemia, heart failure, and death in approximately 10 minutes. As mice consume large quantities of O_2 even at rest (31,33), the fall in PaO_2 and the associated oxyghemoglobin desaturation occurs fairly rapidly (51). Thus, in our experience drawing the ABG after 4 to 10 minutes of single lung ventilation will allow for a sensitive assessment of graft function with reliable and reproducible data obtained by matching the period of single lung ventilation between experimental and control groups.

(IV) Use a 1mL heparin coated syringe with 25 G needle to draw blood from the left ventricle or ascending aorta to measure ABG.

Broncho-alveolar lavage (BAL)

BAL is used to analyze the influx of inflammatory cells into the airways after human lung transplantation (52) and has been used by our laboratory to assess lung grafts (3,53). Using current techniques BAL fluid collection originates from both right and left lung as the lavage is performed with an open chest while intubating the main trachea (*Figure 8*). The advantage of this technique is that it is easy to perform but the disadvantage is that it mixes the airway cell infiltrates of the native and transplanted lung. Nevertheless, our data describe that airway inflammatory cells, such as neutrophils, correlate with tissue infiltration in the transplanted lung (4) and thus we have routinely utilized tracheal BAL to sample the airways and are currently developing methods to use a longer catheter that can be inserted into either the left or right lung selectively. Alternatively one can occlude the native lung in order to collect the BAL sample exclusively from the graft.

Two-photon microscopy

As the lung is constantly exposed to both innocuous and potentially noxious antigens, a thorough understanding of both innate and adaptive immune responses in this organ is essential (30). Two-photon microscopy has evolved into a powerful tool that can allow for observation of cellular interactions in real time. Such a technique has allowed us to substantially extend our understanding of immune responses (54-59). Recently, our group has expanded 2-photon microscopy to the study of the inflammatory responses in the lung, which has allowed us to study ischemia reperfusion injury *in vivo* (28,55,56). By relying on this approach we were able to demonstrate that, contrary to popular belief, monocytes coordinate the transendothelial migration of neutrophils into inflamed tissue. We determined that depletion of blood monocytes impairs neutrophil recruitment to the lung, which could have important implications for the design of therapeutic strategies to treat inflammatory lung diseases (28). Based on this experience, we have expanded the use of intravital two-photon microscopy to investigate cellular trafficking behavior after lung transplantation.

Table 2 ISHLT PGD grading schema

Grade	PaO_2/FiO_2	Radiographic Infiltrates Consistent with Pulmonary Edema
0	>300	Absent
1	>300	Present
2	200-300	Present
3	<200	Present

Time points for assessment: T (0 to within 6 hours of reperfusion, 24, 48, and 72 hours). Data from 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;24:1458.

Scientific uses of mouse orthotopic lung transplantation

Lung transplantation is an established therapy for a variety of end-stage pulmonary disease. Importantly, long-term outcome after lung transplantation are far worse than those of other solid organs (60). Immunologic and non-immunologic mechanisms that contribute to acute and chronic graft lung dysfunction remain poorly understood and the mouse lung transplantation model presents a unique tool that can allow us to study innate and adaptive immune responses after lung transplantation.

Ischemia-reperfusion (I-R) injury-mediated primary graft dysfunction (PGD)

PGD is a form of acute lung injury that results from inflammatory changes induced by I-R injury (61). PGD is graded based on PaO_2/FiO_2 (P/F) ratio and radiographic infiltrates assessed at several time points up to 72 hours after transplantation (*Table 2*). By definition this form of injury arises within the first 72 hours following lung transplantation and is a leading cause of early morbidity and mortality after transplantation. PGD is characterized by impaired oxygenation and pulmonary edema and affects up to 80% of all lung transplant recipients (62-68). In addition, PGD has been linked to the development of chronic allograft rejection manifested by bronchiolitis obliterans (69). Thus, a better knowledge of the pathophysiology of I-R injury should facilitate a better understanding of PGD. Biomarker phenotyping should become possible in order to develop novel therapeutics and reduce the impact of PGD on lung transplant outcomes (62). Neutrophils and factors that control their production and activation play a critical role in I-R injury. Over the last 5 years using the mouse model of orthotopic lung transplantation our group has focused heavily on this cell type. We have delineated that neutrophils isolated from the airways of lung transplantation recipients stimulate donor dendritic cells (DCs) in a contact-dependent fashion to augment their production of IL-12 and expand alloantigen-specific IFN-γ(+) T cells. DC IL-12 expression is largely regulated by degranulation and induced by TNF-α associated with the neutrophil plasma membrane. Extended cold ischemic graft storage enhances G-CSF-mediated granulopoiesis and neutrophilic graft infiltration, resulting in exacerbation of I-R injury after lung transplantation. I-R injury prevents immunosuppression-mediated acceptance of mouse lung allografts unless G-CSF-mediated granulopoiesis is inhibited (19). In addition, we also identified that transcriptional coregulator B cell leukemia/lymphoma 3 (Bcl3) limits granulopoiesis under inflammatory conditions. Bcl3-deficient myeloid progenitors demonstrated an enhanced capacity to proliferate and differentiate into granulocytes following G-CSF stimulation, whereas the accumulation of Bcl3 protein attenuated granulopoiesis in an NF-κB p50-dependent manner (70). Future experiments will focus on therapeutic strategies to modulate the activation of and degranulation of neutrophils in order to ameliorate pulmonary graft injury.

Acute cellular rejection (ACR)

According to the ISHLT Registry, 36% of lung transplant recipients experience at least one episode of ACR within the first year after transplantation (71). Risk factors for ACR remain poorly defined. The degree of major histocompatibility antigen (MHC) discordance between donor and recipient has been identified as a risk factor in some studies (67-69,72). The mouse model of lung transplantation offers an ideal platform to study ACR as inbred mouse strains have well defined MHC antigens allowing for evaluation of ACR in fully mismatched, minor

antigen mismatched, and partially matched (by using F1 crosses as graft donors for example) situations. Since the mouse lung transplantation model was established, we have verified histopathologically and flow cytometrically that this new animal model can recapitulate acute lung allograft rejection successfully (1). We have also demonstrated that, similar to other organs (73), treatment of lung allografts with anti-CD28-B7 and CD40-CD40 Ligand co-stimulatory blockade can prolong allograft survival indefinitely in several strain combinations (7). Dodd-o and colleagues found that anti-CD154 antibody therapy alone is sufficient to attenuate ACR in an MHC mismatched mouse orthotropic lung transplant model. Improved lung allograft acceptance in anti-CD154 Ab treated recipients was associated with abrogated CD8+ and CD4+ allospecific effector responses and increased frequencies of CD4+CD25+Foxp3+ regulatory T-cells in the lung allografts (74). Our group has also provided evidence that pulmonary nonhematopoietic cells, through their expression of MHC-II, play a critical role in downregulating CD4+ T cell-mediated immune responses *in vivo* (2,4). Obviously, this new mouse model will allow for the design of novel studies that elucidate mechanisms of ACR and provide rationale for the development of therapeutic approaches.

Chronic rejection – Obliterative bronchiolitis (OB)

OB is a form of chronic rejection specific to the lung and is characterized by progressive fibrosis and obliteration of the small and medium-sized airways of the donor lung (75). The mechanisms leading to the development of this condition still remain unclear (76). Although lymphocytes are observed in the bronchial wall, the prominent cell type found in the BAL is neutrophils (77). Recently, evidence suggests the involvement of multiple factors such excessive activation of innate immune responses, abnormal angiogenesis and failure of appropriate epithelial regeneration and fibroproliferative tissue remodeling (78). Laboratory experimentation using animal models forms an important component of a "bench-to-bedside-to-bench" approach that can both increase our understanding and lead to the development of novel therapeutic strategies for this (76).

Of the several different animal OB models available, each has advantages and limitations. There is not an "ideal" model that precisely reproduces what happens to humans after lung transplantation. The lesions of OB are thought to represent the shared histological outcome of injury to the airway epithelium and subcellular matrix by an array of immune and inflammatory insults. From a clinical standpoint, OB remains heterogeneous, varying both in timing of onset after transplantation and aggressiveness in clinical course (79). So, it is not practical or scientifically desirable to test multiple contributing factors at the same time. Pulmonary chronic rejection shares features of chronic rejection observed in other solid organ. Chronic rejection in all organs mainly manifests as fibrosis in the graft resulting in loss of function and eventually grafts loss (80). There are several immunologic antigens involved in this fibrosis, including major and minor histocompatibility antigens (81) as well as self-antigens (82) as both can emerge as targets of immune responses after transplantation (83-85).

In theory the orthotopic mouse lung transplantation model has great advantages for studying the pathogenesis of OB as it offers a physiological model of the human lung transplant environment. Lung function and BAL can be assessed at various times, various drugs can be tested for efficacy, and transgenic and knock-out strains are available to model human diseases. However, several physiologic factors in the mouse lung anatomy and physiology create potential obstacles in the study of OB. The initial OB process starts with a lymphocytic infiltrate of the sub-mucosa of the airways followed by the migration of the lymphocytes through the basement membrane into the epithelium (86). At this site, epithelial cell necrosis occurs with denudation of mucosa. In the mouse lung, there is lack of submucosal glands in the bronchioles, but a high numbers of locally resident Clara cells. One of the main functions of Clara cells is to protect the bronchiolar epithelium from injury. They accomplish this by secreting a variety of proteins, including Clara cell secretory protein (CCSP) and a solution similar to lung surfactant. They are also responsible for detoxifying inhaled harmful substances. Clara cells also act as a stem cell and multiply to differentiate into ciliated cells that can regenerate bronchiolar epithelium. This is a possible reason why mouse airway epithelium remains intact and fully differentiated in lung allografts, despite profound vascular rejection (7). Since 2007, a few separate groups have tried to establish an OB model in the mouse. Although two groups have found OB lesions by histology, the experimental design and the histological features still need further clarification (9,27). Currently we are focusing on three factors that may influence the development of OB including: (I) donor and recipient strain combinations; (II) time period necessary to see chronic rejection in the mouse and; (III) the form of immunosuppression that may allow for OB to develop.

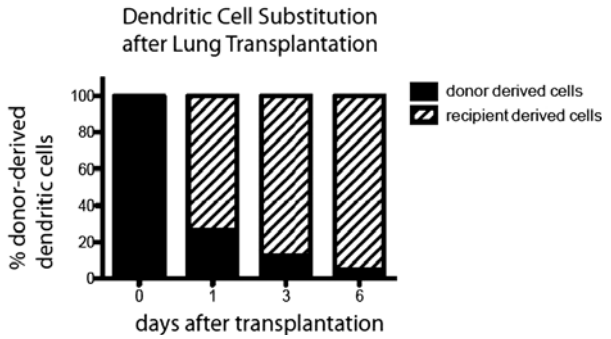

Figure 9 Rapid substitution of professional antigen presenting cells, such as dendritic cells, occurs after orthotopic lung transplantation.

Once established and reproducible, such a model could hold great promise for further mechanistic studies and may be used to accelerate the development of new strategies for the prevention or treatment of pulmonary chronic rejection.

Studies of Non-hematopoietic stromal cells

While bone marrow-derived hematopoietic cells, such as dendritic cells, play a critical role in pulmonary physiology (87-92), we as well as others have focused on the contribution of non-hematopoietic cells in pulmonary immune responses (4,93). Traditional methods for separating the physiology of hematopoietic from non-hematopoietic cells involves the creation of bone marrow chimeras by lethal irradiation of a recipient mouse followed by reconstitution with bone marrow from a mutant strain (94). Donor irradiation, however, may change the physiology of non-hematopoietic cells and bone marrow chimeras can also suffer from autoimmune disease (4). We have recently demonstrated that the transplantation of a left lung into a congenic host leads to the rapid substitution of donor-derived hematopoietic cells with those of the host, leading to the creation of a "chimeric lung graft" in an otherwise immunocompetent host. Furthermore, such substitution occurs for multiple types of hematopoietic cells such as T cells (4,5) and dendritic cells (*Figure 9*). Such a model can thus be used to study both immunology and physiology of pulmonary non-hematopoietic cells in the absence of irradiation.

Conclusions

Although technically difficult, orthotopic lung transplantation in the mouse is feasible and reproducible. It has been used for multiple scientific avenues of investigation for the last 5 years by our laboratory as well as others. Obviously, it is important for researchers to understand the physiology behind this model as well as its advantages and limitations. Well-designed mouse orthotopic lung transplantation experiments provide a great opportunity to study mechanisms that affect lung allograft survival and explore new therapies for a wide variety of human lung diseases.

Acknowledgements

Funding: This study was supported by American Thoracic Society/Lungevity Foundation, NIH 1R01HL094601, the Barnes Jewish Research Foundation, The American Association for Thoracic Surgery and the Thoracic Surgery Foundation for Research and Education.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Lin X, Li W, Lai J, Okazaki M, Sugimoto S, Yamamoto S, Wang X, Gelman AE, Kreisel D, Krupnick AS. Five-year update on the mouse model of orthotopic lung transplantation: Scientific uses, tricks of the trade, and tips for success. J Thorac Dis 2012;4(3):247-258. doi: 10.3978/j.issn.2072-1439.2012.06.02

Surgical technique for lung retransplantation in the mouse

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Abstract: Microsurgical cuff techniques for orthotopic vascularized murine lung transplantation have allowed for the design of studies that examine mechanisms contributing to the high failure rate of pulmonary grafts. Here, we provide a detailed technical description of orthotopic lung retransplantation in mice, which we have thus far performed in 144 animals. The total time of the retransplantation procedure is approximately 55 minutes, 20 minutes for donor harvest and 35 minutes for the implantation, with a success rate exceeding 95%. The mouse lung retransplantation model represents a novel and powerful tool to examine how cells that reside in or infiltrate pulmonary grafts shape immune responses.

Keywords: Lung transplantation; mice; experimental models

Submitted Apr 15, 2013. Accepted for publication Apr 20, 2013.
doi: 10.3978/j.issn.2072-1439.2013.04.15
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.04.15

Introduction

Physiologically relevant animal models are critical to advance our understanding of graft failure after lung transplantation in humans. Our laboratory has developed techniques for orthotopic vascularized lung transplantation in the mouse, which have allowed for the design of studies that examine mechanisms contributing to the high failure rate of pulmonary grafts (1,2). Retransplantation of various tissues has been utilized to study how graft-resident or -infiltrating cells regulate immune responses to specific grafts (3,4). We have recently developed and validated a method to retransplant mouse lungs, which we used to investigate the role of early alloimmune responses in pulmonary allograft rejection and acceptance (5). Here we provide a detailed technical description for left pulmonary retransplantation in the mouse utilizing cuff techniques that represents an addition to the available experimental models in lung transplantation.

Operation techniques

Left orthotopic vascularized lung transplants are performed in syngeneic or allogeneic strain combinations as previously described (1). Donor and recipient mice are anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. At various time points ranging from 3 to 30 days after the initial transplant the lung grafts are harvested from these hosts. Animal procedures were approved by our institutional Animal Studies Committee.

Donor operation

Anesthetized mice are intubated with a 20-gauge angiocatheter and connected to a ventilator with room air at a tidal volume of 0.5 mL and a respiratory rate of 110-120/min. 100 units of heparin are injected intravenously before the harvest. Left lung grafts are exposed through a median sternotomy and flushed with 3 mL of cold low-potassium dextran glucose (LPDG) solution through the main pulmonary artery after dividing the inferior and superior venae cavae. While harvesting the heart-lung block from the initial host, dissection needs to be performed carefully due to the formation of adhesions between the graft and the chest wall, which we typically encounter at the site of the original incision. With increased experience we found

that in some cases, if adhesions are severe, it is preferable to leave a small piece of chest wall tissue on the surface of the graft to prevent injury to the graft, which could result in pneumothoraces. The heart-lung block is excised and the lung graft is prepared for retransplantation in a petri dish filled with ice cold LPDG solution (*Figure 1A,E*). The left hilum is dissected and the cuffs, which had been placed during the initial transplant, are exposed (*Figure 1B,F*). The 10-0 nylon suture ligatures, which had been used to secure the cuffs to the respective recipient vessels, are removed (*Figure 1C,G*). We recommend the use of 10-0 nylon to secure the cuffs to the recipient structures during the initial transplant because of the relative ease of the removal of monofilament as compared to braided suture material. Caution is required when removing these suture ligatures to avoid the accidental severing of the ligatures that have been used to secure the cuffs to the donor structures. The pulmonary vein and artery are cut proximal to the previously placed cuffs (24 G on pulmonary artery and 20 G on pulmonary vein). Subsequently the recipient vessels are dissected from the graft while maintaining the original cuffs (*Figure 1D,H,I,M*). Particularly when we perform the retransplant procedure at extended time periods after the initial engraftment (e.g., 30 days), it has been our experience that dense adhesions often prevent us from separating the recipient vessels from the donor vessels after release of the ligature. Under these circumstances, we divide the recipient vessel distal to the cuff, fold it over the cuff and secure it to the cuff with a 10-0 nylon tie. We then flush the lung graft with 1 mL of LPDG solution through the pulmonary artery (*Figure 1J,N*) and subsequently store the heart-lung block in LPDG solution at 4 ℃. During this time period the graft bronchus remains cuffed (18 G) and attached to the recipient bronchus (*Figure 1K,O*). To prevent preservation solution from entering the airway during storage, the suture ligature that had been placed during the initial transplant procedure to secure the recipient to the donor bronchus is not released until the graft is retransplanted (*Figure 1L,P*).

Recipient operation

Secondary recipient mice are anesthetized, intubated orotracheally and placed in a right lateral decubitus position. A left thoracotomy is performed through the third intercostal space. A clamp is attached to the native left lung for lateral retraction and exposure of the hilum (*Figure 2A,E*). The hilum of the left lung is dissected and the pulmonary artery and vein are occluded temporarily with a slip knot (8-0 silk suture) (*Figure 2B,F*).

The left main bronchus is occluded with a microvascular clip (*Figure 2C,G*). An incision is then made in each of these structures at the same level in preparation for the insertion of the cuffed donor structures (*Figure 2D,H*). At this point we separate the bronchus of the lung graft from the bronchus of the initial recipient (*Figure 1K,O*). As we have described for the vessels above, in case we encounter severe adhesions between the bronchus of the initial recipient and the cuff on the donor bronchus we cut the recipient bronchus distal to the cuff, fold the bronchus over the cuff and secure it with an additional 10-0 nylon tie. Cuffed donor pulmonary artery, vein and bronchus are inserted into the respective recipient structures and the cuffs are secured with 10-0 nylon suture ligatures (*Figure 2I,J,K,M,N,O*). Close attention needs to be paid to maintaining the proper orientation of the hilar structures during reimplantation of the graft to prevent their torsion, which can result in technical failure. This may necessitate lysis of adhesions within the graft hilum and dissection of the vessels and bronchus. The graft is reperfused and ventilated upon release of the ties and clip that had occluded the recipient structures (*Figure 2L,P*). The chest incision is closed in two layers with 6-0 nylon and the mouse is extubated.

The total time of the retransplantation procedure is approximately 55 minutes, 20 minutes for the donor procedure and 35 minutes for the reimplantation. Warm ischemic times are comparable to primary lung transplants. To date, we have completed 144 retransplantation procedures with a technical success rate exceeding 95%. In syngeneic combinations, retransplanted grafts remain ventilated and free of inflammation for at least 3 months after retransplantation, similar to primary transplants (*Figure 3*).

Comments

Experiments using rodent models of graft retransplantation have yielded important insights in transplantation biology. For example, the immunogenic role of passenger leukocytes has been defined in organ parking experiments using re-transplanted rat kidneys (3). Retransplantation of lungs has also been reported in the rat model (6). Compared to rats, however, mice offer important advantages such as abundant transgenic strains and availability of reagents.

Cuff anastomotic techniques have been widely used

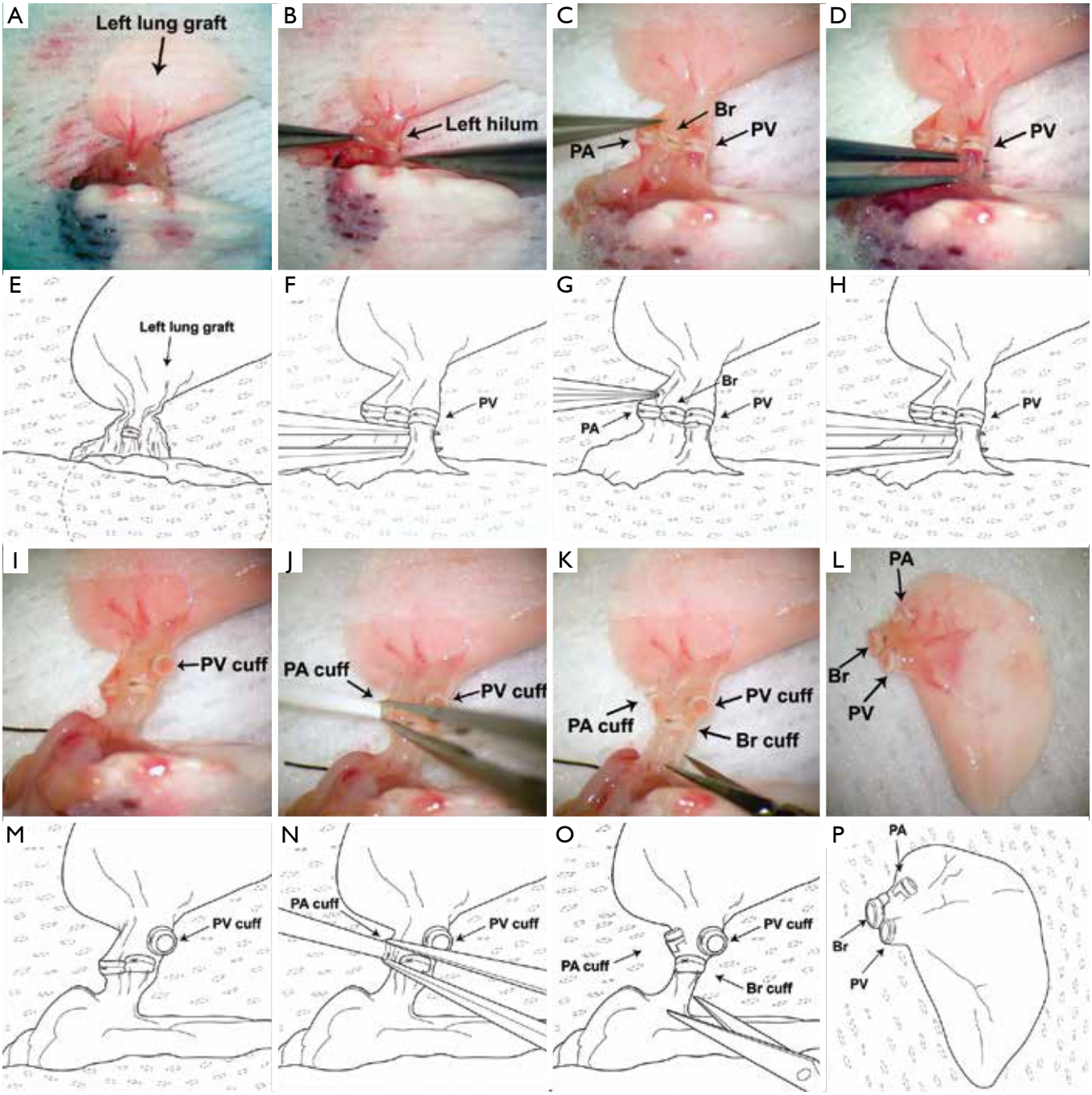

Figure 1 Preparation of left lung graft for retransplantation. (A,E), The heart-lung block is excised from the initial host and the hilar structures of the left lung graft are exposed; (B,F), The left hilar structures are dissected and the cuffs exposed; (C,G), The ligature that had been placed on the pulmonary vein (denoted as PV) during the initial transplant is removed; ("PA" denotes the pulmonary artery; "Br" denotes the bronchus.); (D,H), The pulmonary vein is divided proximal to the previously placed cuff; (I,M), The pulmonary vein is dissected leaving the original cuff in place; (J,N), The lung graft is flushed with 1 mL of ice-cold LPDG solution through the pulmonary artery; (K,O), Cuffs on pulmonary artery and vein are released with the graft bronchus remaining attached to the initial recipient; (L,P), The suture ligature that had been placed on the bronchus during the initial transplant is released before reimplantation.

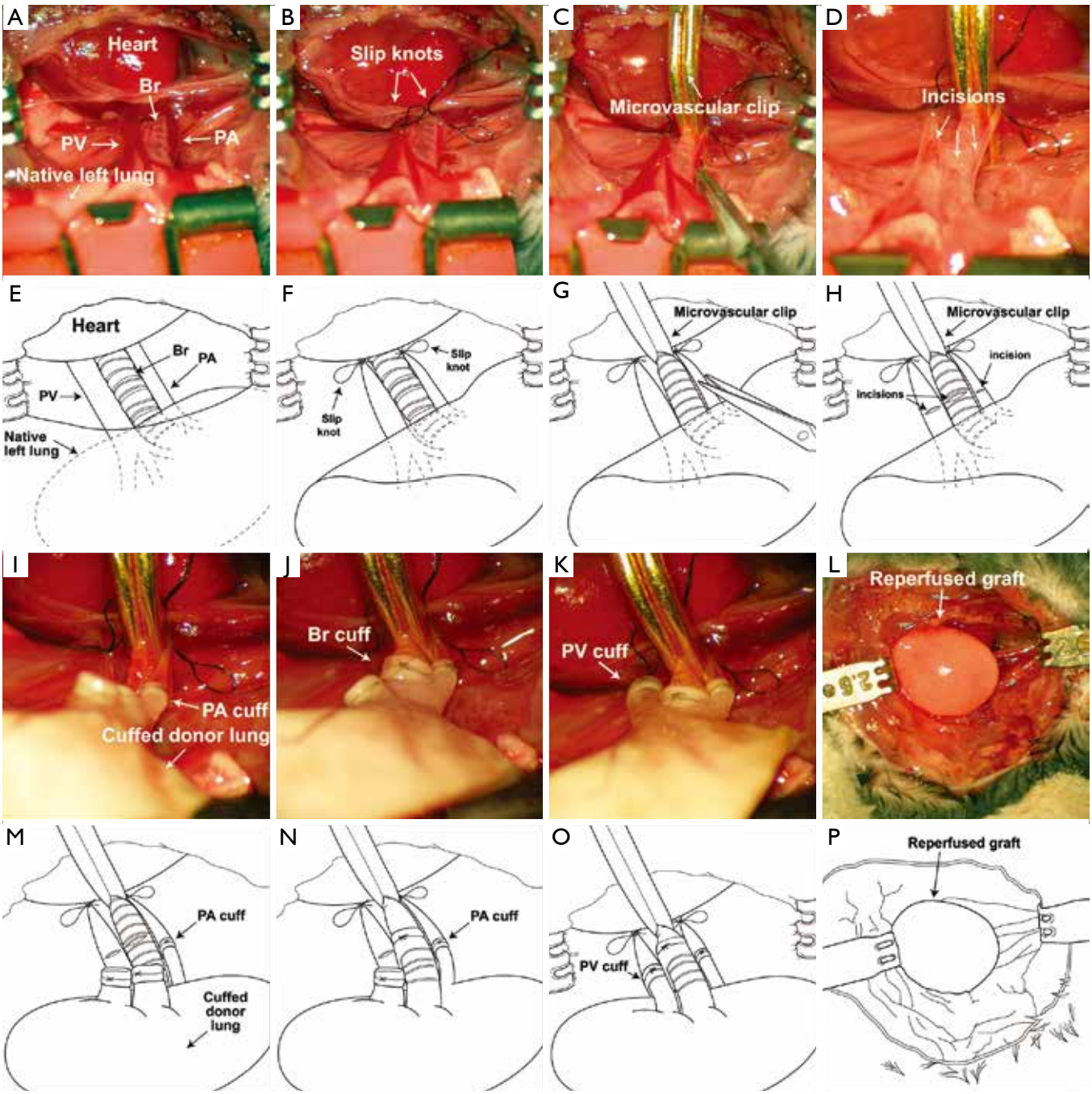

Figure 2 Retransplantation of left lung graft. (A,E), A clamp is attached to the native left lung for lateral retraction and hilar exposure; (B,F), Pulmonary artery and vein are occluded temporarily with a slip knot; (C,G), The left main bronchus is occluded with a microvascular clip; (D,H), Incisions are made in pulmonary artery, pulmonary vein and bronchus; (I,M), Cuffed donor pulmonary artery; (J,N), donor bronchus and (K,O), donor pulmonary vein are inserted into the respective recipient structures and secured with 10-0 nylon ligatures; (L,P), The graft is reperfused and ventilated upon release of the ties and clip.

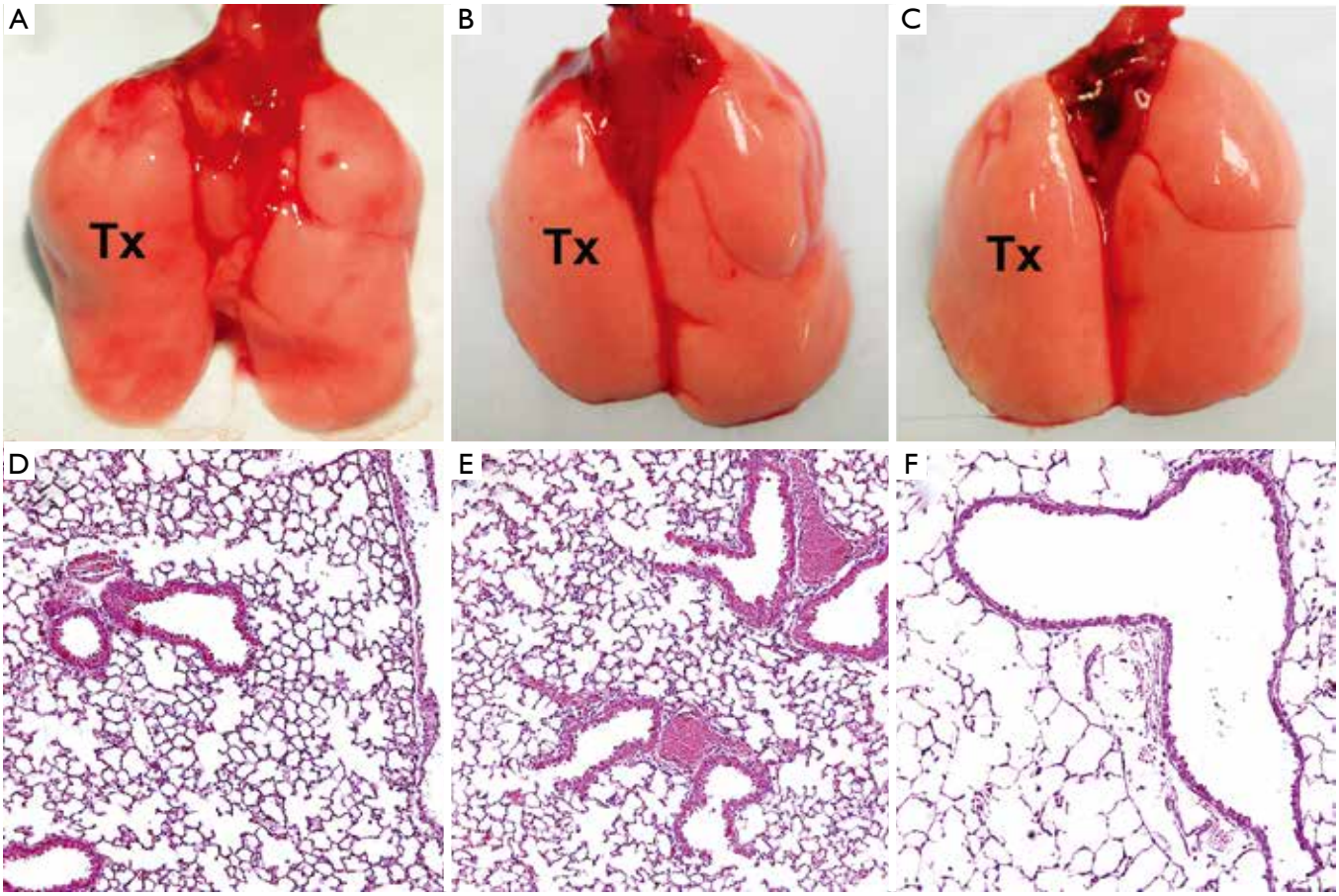

Figure 3 Gross appearance and histology (H&E) (100×) of (A,D) C57BL/6 (B6) lungs transplanted into syngeneic hosts 90 days after engraftment (Tx denotes transplanted left lung). Gross appearance and histology (H&E) (100×) of B6 lungs initially transplanted into syngenic host for (B,E) 4 days or (C,F) 30 days and then retransplanted into second syngeneic host shown 90 days after retransplantation.

for the transplantation of a variety of rodent organs. The use of cuff techniques that we have described for primary lung transplants has allowed us to retransplant mouse lungs. Employing this technique has recently allowed us to compare the role of early alloimmune responses in rejection and acceptance of lung and heart grafts (5). We found that, unlike the case for hearts, a short period of immunosuppression establishes regulatory pathways within lung grafts that allow them to survive for extended periods of time in nonimmunosuppressed allogeneic hosts.

The mouse lung retransplantation model represents a novel and powerful tool to examine how cells that reside in or infiltrate pulmonary grafts regulate immune responses. The importance of this model is highlighted by our recent demonstrations that the immune response to lung grafts differs from other organs (5,7).

Acknowledgements

We thank Arlene Ligori for medical illustration.
Funding sources: D.K. and A.E.G. are supported by a grant sponsored by The National Heart, Lung, and Blood Institute (1R01HL094601). D.K. and A.S.K. are supported by a grant sponsored by The National Heart, Lung, and Blood Institute (NIH R01 HL113931).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Li W, Goldstein DR, Bribriesco AC, Nava RG, Spahn JH, Wang X, Gelman AE, Krupnick AS, Kreisel D. Surgical technique for lung retransplantation in the mouse. J Thorac Dis 2013;5(3):321-325. doi: 10.3978/j.issn.2072-1439.2013.04.15

Lung donor selection criteria

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Abstract: The criteria that define acceptable physiologic and social parameters for lung donation have remained constant since their empiric determination in the 1980s. These criteria include a donor age between 25-40, a arterial partial pressure of oxygen (PaO_2)/FiO_2 ratio greater than 350, no smoking history, a clear chest X-ray, clean bronchoscopy, and a minimal ischemic time. Due to the paucity of organ donors, and the increasing number of patients requiring lung transplant, finding a donor that meets all of these criteria is quite rare. As such, many transplants have been performed where the donor does not meet these stringent criteria. Over the last decade, numerous reports have been published examining the effects of individual acceptance criteria on lung transplant survival and graft function. These studies suggest that there is little impact of the historical criteria on either short or long term outcomes. For age, donors should be within 18 to 64 years old. Gender may relay benefit to all female recipients especially in male to female transplants, although results are mixed in these studies. Race matched donor/recipients have improved outcomes and African American donors convey worse prognosis. Smoking donors may decrease recipient survival post transplant, but provide a life saving opportunity for recipients that may otherwise remain on the transplant waiting list. No specific gram stain or bronchoscopic findings are reflected in recipient outcomes. Chest radiographs are a poor indicator of lung donor function and should not adversely affect organ usage aside for concerns over malignancy. Ischemic time greater than six hours has no documented adverse effects on recipient mortality and should not limit donor retrieval distances. Brain dead donors and deceased donors have equivalent prognosis. Initial PaO_2/FiO_2 ratios less than 300 should not dissuade donor organ usage, although recruitment techniques should be implemented with intent to transplant.

Keywords: Lung transplant; donor criteria; review

Submitted Feb 04, 2014. Accepted for publication Mar 18, 2014.
doi: 10.3978/j.issn.2072-1439.2014.03.24
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.03.24

Introduction

Lung transplantation is an established therapy for selected patients with end-stage pulmonary disease. Since the first successful lung transplant in 1983 by Dr. Joel Cooper and his team, over 42,000 recipients have benefitted from this procedure worldwide. Advances in surgical techniques, postoperative care, and immunosuppression therapy have led to improved short- and long-term survival following lung transplantation. Despite this success, the number of suitable lung donors remains a significant limitation. Today many donors are judged based on empiric criteria developed in the 1980s (See *Table 1*) (2,3).

Most centers agree that these criteria are too strict and use extended criteria donors (ECD) that do not completely meet the traditional empiric criteria (4). Many centers advocate use of ECD to effectively increase the donor pool with similar transplant outcomes (2,5-10). There is considerable variation in practice patterns among these centers and no uniformly accepted discriminating metric (6).

In-hospital mortality for lung transplantation is higher than for other solid organs. A significant contributor to this early hazard is primary graft dysfunction (PGD) (11). PGD occurs in up to 25% of recipients with associated 30 days

Table 1 Ideal lung donor criteria (1)	
Age	20-45
PaO_2:FiO_2	>350
Smoking history	None
Chest X-ray	Clear
Ventilation days	<5
Microbiology	Gram stain negative
Bronchoscopy	Clear
Ischemic time	<4 hours

mortality of 40-50%; compared to 5-10% without PGD (12). Accumulating evidence suggests that PGD is the end result of a series of injuries occurring in the donor lung from the time of brain death to reperfusion in the recipient (13). Therefore, concern over PGD may drive concern over lung donors, and thus limit the number of organs considered usable for transplant. Given the increasing burden of lung disease, the extremely limited number of suitable lung donors, and increasing waitlist mortality, it is not surprising that an increasing numbers of ECDs are being used. In the era of the lung allocation score, with preferential allocation to sicker recipients, it becomes more important to understand not only which ideal criteria can be ignored, but also in which context. Here, we break down donor criteria by individual factors and examine their effect on outcomes.

Age

Over the last 30 years, the average age of donors accepted for transplant has steadily increased. Retrospective cohort analysis of OPTN data revealed no increases in one year graft failure with donors aged 18-64. Ages <18 and >64 were associated with increased failure rates at one year but were not associated with increased PGD (14). Retrospective review of UNOS data from 2000-2010 confirms an increase in 1- and 3-year mortality for donors over the age of 65 without increases in bronchiolitis obliterans syndrome (BOS) (15). Further stratification into age groups [50-54, 55-59 and 60-64] did not reveal differences in one year mortality or FEV_1 (16). Available literature favors consistent outcomes for donors within the range of 18-64 years.

Gender

Donor and recipient gender combinations have been analyzed with mixed results. Fessart *et al.* failed to discern a difference in recipient survival after analysis of all gender combinations (17). Another single center retrospective study demonstrated an increase in survival and decrease in BOS for donor recipient gender mismatches (M→F and F→M). Male donor to male recipients specifically had a significant decrease in survival (18). International Society of Heart and Lung Transplant (ISHLT) registry review from 1995-2002 reflected a decreased survival in female donors to male recipients. Female donor to female recipient demonstrated a short and long term survival benefit (19). These results coincided with a multicenter study in France (20). The exact gender interactions between donor and recipient have yet to be defined to accurately shape our practice of transplant selection. There are questionable effects of hormones and size mismatch that have yet to be delineated in the literature.

Race

Retrospective review of lung transplants from 1997 to 2007 of race matched donors and recipients conferred a 3.3% decreased risk adjusted mortality at five years and 12% overall mortality in recipients with cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF) and single lung transplant (SLT). No changes in one year rejection rates were associated with race matching. Donor African American lungs reflected an increased risk of death regardless of recipient. Overall, specific recipient race was not associated with survival variability (21).

Smoking history

In the UK, a smoking history in donor lungs is associated with decreased recipient survival as compared to non-smoker donor lungs. The recipient survival, however, remains greater than that of the wait list population (22). This raises the argument that patients with high mortality risk would benefit from transplantation rather than succumb to illness on the waiting list. The interpretation of this data is also limited given recipients of smoker lungs were riskier candidates prior to surgery. Smoker donor lungs confer a higher risk of grade 3 PGD (23). A retrospective review of UNOS data on 766 heavy smoker donor lungs (>20 pack year history) revealed no increases in BOS or median survival (24). An additional single retrospective study of smoking donors revealed a worse early survival but no effect on long term survival and BOS incidence (25). This was confirmed by an additional retrospective single institution study that had

prolonged postoperative intubation and ICU stay in smokers but equivalent survival at three years (26). The overall findings coincide with an initial higher postoperative risk, and equivalent to higher long term recipient mortality risk, for smoker donor lungs as compared to non-smoker donor lungs. The mortality of patients receiving smoker donor lungs does reflect a lower mortality risk than that of patients on the transplant waiting list.

Bronchoscopic findings and cultures

Post transplantation pneumonia and sepsis are serious concerns to the transplant surgeon and previous guidelines for chest X-ray and bronchoscopy attempt to avoid transmission to immunosuppressed recipients. Gram stain evaluation of airways in a single center retrospective study found 12% of donors with a positive gram stain subsequently developed recipient pneumonia while 20% of negative gram stain donors went on to develop pneumonia. This refutes the association of donor gram stain with recipient pneumonia. In this study, however, donor lungs were not accepted if there was evidence of frank aspiration on bronchoscopy (27). Prospective analysis of donor airway cultures and bronchial tissue cultures revealed a <1.5% transmission rate of donor organ contamination (28). The lack of infection transmission from donor to non-suppurative based recipients is also been confirmed by two separate studies (29,30). With appropriate antibiotic prophylaxis to cover Pseudomonas and Staph aureus, risk of transmission of donor associated infection is negligible.

Radiographic findings

Donors undergo multiple radiographs prior to surgery. The high degree of interpretation variability have diminished the role in donor selection criteria (31). One third of possible donor radiographs in a retrospective survey had infiltrates, of which greater than half improved or spontaneously resolved. Improvement in infiltrates did not impact transplantation rates and led to unnecessary rejection. All patients transplanted in this study with positive infiltrates were alive at one year follow-up (32). No studies were found that correlated chest radiograph findings to recipient infections. The literature on radiographic donor exclusion is extremely limited, and the topic warrants further investigation.

Size mismatch

A recent review by Barnard published in 2013 thoroughly outlines size criteria for donor/recipient, and their results are briefly summarized here (33). Total lung capacity (TLC), recipient pathology (obstructive *vs.* restrictive), and height all factor in to appropriate matches. For double lung transplants, patients with emphysema should be matched to a donor with a 67-100% of the recipient's TLC. No definitive data is available for SLT for emphysema. For pulmonary hypertension and CF patients, the predicted total lung capacity (pTLC) of the donor may safely reach 120% of the recipient actual TLC. Due to the limitations in TLC that occur in pulmonary fibrosis, the recommendation for donors pTLC is to be within 20% of the halfway point between the recipients actual TLC and pTLC. For SLT for fibrotics, the donor pTLC should be within 20% of the recipient's pTLC. Little data exists for transplantation in overt size mismatch, but some suggest it is preferable to slightly oversize if possible and not undersize less than 80% (34).

Ischemic time and donor distance

Retrospective review of UNOS data of 6,055 transplants revealed no increased incidence of BOS or three years mortality in recipients with local, regional or national lung donors despite national ischemic times of (342±90) minutes (35). Additional single center studies verify no change in survival for ischemia greater than six hours (36-40). Donor ischemia time >7 hours and donor age >50 years compounded, however, was associated with decreased recipient survival at two years (41).

Donation after cardiac death

After evaluating the literature for effects of ischemia on recipient outcomes, the question of donation after cardiac death (DCD) use as opposed to beating heart brain dead donors inevitably follows. The largest single center study with 409 DCD lungs revealed a decrease in graft survival that did not reach statistical significance. The patient survival and BOS were comparable (42). Smaller, single center studies reveal either similar survival rates (43,44), or a modest decrement in survival (45). A single institutional study out of Madrid revealed PGD in 72%, Survival rates of 51% at five years, and BOS of 45% at five years (46). Use

of DCD donor lungs revealed a 100% survival at almost a year in eight patients (47). In total, these studies suggest the benefit of using DCD donors as a means to expand the available donor pool.

High risk donors

The Centers for Disease Control and Prevention (CDC) label high risk donors as those with exposure to HIV, prison inmates, IV drug users, prostitution history, high risk sexual history, and hemophiliacs. Limited data is available for lung transplantation in CDC high risk donors. Review of UNOS database on CDC high risk donors demonstrated equivalent one year mortality, postoperative infection, stroke and dialysis with normal donors. Around 9% of lung donors were classified as high risk and risk of disease transmission was less than 1%. Interestingly 95% of recipients surveyed would accept an organ from a high risk donor with an expected donor pool expansion of 10% (48).

Oxygenation

Arterial partial pressure of oxygen (PaO_2) is a traditional way to measure lung function. Donors with initial PaO_2/FiO_2 of <300, that improved to >300 with recruitment maneuvers, used in Australia were not associated with a decreased 30 days, 1, 2, 3 yrs survival or recipient PaO_2/FiO_2 ratio (8). High dose steroid administration after brain death was associated with an increase in PaO_2/FiO_2 of 16 +/-14 and a decrease of 34.2 +/-14 if steroids were not given. The outcome of recipients receiving steroid treated donor lungs was not analyzed in this study (49). Most importantly, UNOS data from 2000 to 2009 of 12,045 transplants failed to demonstrate a PaO_2 association with decreased survival, even with a PaO_2 of less than 200 in 1,830 patients (50). This may be due to preoperative gasses that are lower on initial reported PaO_2 and significantly improve after recruitment maneuvers, which are not consistently captured in the database.

Ex vivo lung perfusion (EVLP)

EVLP is an emerging technique used to evaluate and potentially salvage high-risk donor organs typically not suitable for lung transplantation (51). Steen initially utilized this technique to evaluate a DCD donor (52) and their success has sparked several studies around the world (51,53-57). These studies have demonstrated similar length of mechanical ventilation, rate of PGD, length of stay and mortality. How this technology will be implemented in allocation has yet to be determined despite the considerable promise they imply. Despite these challenges, it appears that the future of lung transplantation will capitalize on EVLP to safely expand the donor pool by expanding the limits of what defines a suitable donor.

Conclusions

There is little data to suggest that any of the historical criteria for defining the ideal lung transplant donor impact either short or long term outcomes. For age, donors should be within 18 to 64 years old. Gender may relay benefit to all female recipients especially in male to female transplants. Negative outcomes are associated with female donors to male recipients. Race matched donor/recipients have improved outcomes and African American donors convey worse prognosis. Smoking donors may decrease recipient survival post transplant, but provide a life saving opportunity for recipients that may otherwise remain on the transplant waiting list. No specific gram stain or bronchoscopic findings are reflected in recipient outcomes. Chest radiographs are a poor indicator of lung donor function and should not adversely affect organ usage aside for concerns over malignancy. Ischemic time greater than six hours has no documented adverse effects on recipient mortality and should not limit donor retrieval distances. Brain dead donors and deceased donors have equivalent prognosis. Initial PaO_2/FiO_2 ratios less than 300 should not dissuade donor organ usage, although recruitment techniques should be implemented with intent to transplant.

Although there have been multiple trials on individual lung donor criteria that fail to show negative recipient prognosis (58), there are few studies that evaluate the effects of multiple extended criteria compounded together in one donor lung. These compromises in physiology may have untold effects on PGD and overall patient mortality. In additional to donor selection, it is imperative to consider the recipient's pathology as a major harbinger of overall transplantation outcome (59). It is currently our recommendation that any single criteria outside of the historical ideals can safely be ignored, but we caution that the cumulative effects of multiple extended donation criteria in one donor have not been studied.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors declare no conflict of interest.

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Cite this article as: Chaney J, Suzuki Y, Cantu E III, van Berkel V. Lung donor selection criteria. J Thorac Dis 2014;6(8):1032-1038. doi: 10.3978/j.issn.2072-1439.2014.03.24

Indications and outcomes in adult lung transplantation

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Abstract: Lung transplantation (LTx) is a treatment option for end-stage lung disease that would be otherwise fatal for specific patient populations. The most common indications for LTx in adults remain to be chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, cystic fibrosis, alpha-1 antitrypsin deficiency, and idiopathic pulmonary arterial hypertension. Recent trends include performing re-transplantation while more patients over the age of 65 years are undergoing LTx. Even with these tendencies, slight improvements in survival have occurred. This article briefly reviews recent developments in adults undergoing LTx.

Keywords: Adults; indications; outcomes; lung transplantation (LTx)

Submitted Jun 21, 2014. Accepted for publication Jun 23, 2014.
doi: 10.3978/j.issn.2072-1439.2014.07.04
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.07.04

Introduction

Lung transplantation (LTx) is the only therapeutic option for end-stage parenchymal lung diseases or pulmonary vascular disorders. In 1963, Hardy *et al.* (1) performed the first lung transplant in a 58-year-old male patient who died of nephrotoxicity. Since then, significant advancements have occurred regarding organ preservation, extracorporeal support of both donor organs and recipients, surgical techniques, immunosuppressive therapeutic agents, and allograft surveillance, along with the advent of multidisciplinary, collaborative medical and surgical teams to provide care to patients after LTx. The purpose of this brief review is to review indications for LTx in adult patients and to present clinical outcomes.

Recent trends in lung transplant numbers

The International Society for Heart and Lung Transplantation (ISHLT) Registry provides detailed annual information on patients who have undergone LTx. The most recent report in 2013 summarized data from 43,428 adult lung and 3,703 adult heart-lung transplant recipients and their donors through June 30, 2012 (2). The number of lung transplants has continued to rise, especially over the last 5 years (*Figure 1*); however, this increase in demand for organs has coincided with a reduction in number of available donor lungs (2,3). Coinciding with the increase in total lung transplants, patients who are older than 65 years undergoing LTx are on the rise (*Figure 1*) (2,3). Similarly, the age of donor lung allografts is on the rise (4).

Indications for lung transplantation (LTx) in adults

The decision to perform LTx is a complex treatment that carries considerable surgical risks. *Table 1* shows the indications for lung transplants in adults performed between January 1995 and June 2012, while *Figure 2* provides the major indications by year from 1990 to 2011 (2). Revision of international guidelines for lung transplant candidates was last published in 2006 by Orens *et al.* (5) with a revised update being published soon, which will include pediatric recommendations for the first time.

Table 2 lists the major disease categories that should be considered for LTx. Patients with these pulmonary disorders should be referred for consideration for LTx at any point if these characteristics exist or if the patient or primary healthcare provider has further questions regarding

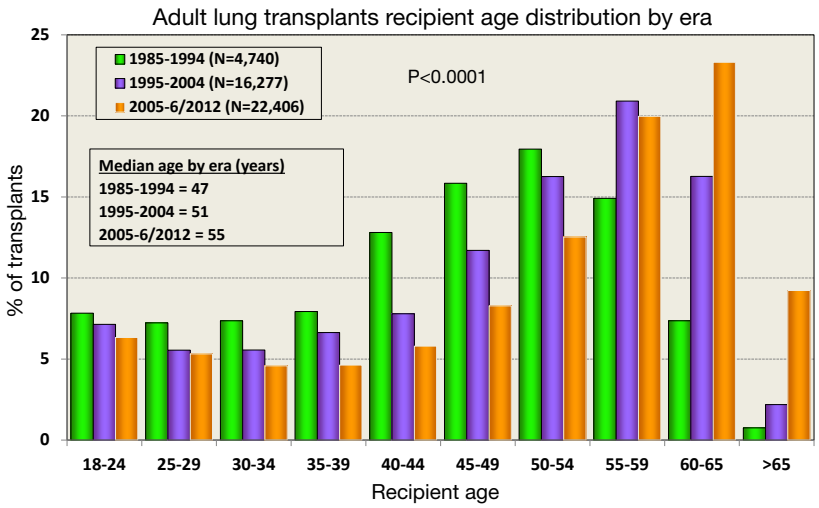

Figure 1 Major indications for lung transplants by year (%) from 1990 to 2011, modified with permission (2). The age distribution of lung transplant recipients was compared between eras using a chi-square test. A significant P value means that at least one of the groups is different than the others but it doesn't identify which group it is.

Table 1 Indications for adult lung transplants between January 1995 to June 2012, modified with permission (2)

Diagnosis	Single lung (N=14,197) No. (%)	Bilateral lung (N=23,384) No. (%)	Total (N=37,581) No. (%)
COPD* (without alpha-1 antitrypsin deficiency)	6,312 (44.5)	6,290 (26.9)	12,602 (33.5)
COPD* (with alpha-1 antitrypsin deficiency)	753 (5.3)	1,429 (6.1)	2,182 (5.8)
Interstitial lung disease (with idiopathic pulmonary fibrosis)	4,872 (34.3)	4,032 (17.2)	8,904 (23.7)
Bronchiectasis associated with cystic fibrosis	229 (1.6)	6,002 (25.7)	6,231 (16.6)
Idiopathic pulmonary arterial hypertension	87 (0.6)	1,073 (4.6)	1,160 (3.1)
Pulmonary fibrosis, other	563 (4.0)	820 (3.5)	1,383 (3.7)
Bronchiectasis	59 (0.4)	956 (4.1)	1,015 (2.7)
Retransplant (obliterative bronchiolitis)	276 (1.9)	292 (1.2)	568 (1.5)
Retransplant (not obliterative bronchiolitis)	182 (1.3)	220 (0.9)	402 (1.1)
Sarcoidosis	265 (1.9)	689 (2.9)	954 (2.5)
Connective tissue disease	156 (1.1)	332 (1.4)	488 (1.3)
Obliterative bronchiolitis (not retransplant)	98 (0.7)	298 (1.3)	396 (1.1)
Lymphangioleiomyomatosis	136 (1.0)	255 (1.1)	391 (1.0)
Congenital heart disease	56 (0.4)	269 (1.2)	325 (0.9)
Cancer	7 (0.0)	29 (0.1)	36 (0.1)
Other	146 (1.0)	398 (1.7)	544 (1.4)

*, COPD, chronic obstructive pulmonary disease.

the potential benefit of LTx. *Tables 3,4* outlines both absolute and relative contraindications for LTx as recently recommended. In short, LTx should not be considered in a patient with a florid infection, recent malignant tumor, continued addictive behavior, or lacks reliable social support. Infectious issues are different in cystic fibrosis with controversy continuing with most centers generally not offering transplant in patients colonized with *Burkholderia*

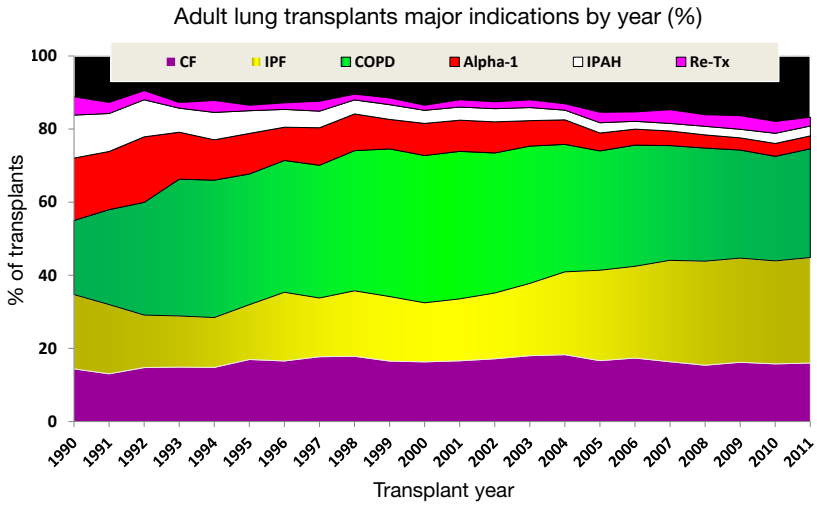

Figure 2 Adult lung transplants recipient age distribution by era from 1985 to 2012, modified with permission (2).

Table 2 Indications for lung transplantation according to underlying major diseases
Chronic obstructive pulmonary disease (with or without alpha-1 antitrypsin deficiency)
BODE (body-mass index, airflow obstruction, dyspnea, and exercise) index >5
FEV_1 <20% of predicted
Diffusion capacity <20% of predicted
Pulmonary hypertension or cor pulmonale despite oxygen therapy
Hypercapnia, P_aCO_2 >50 mmHg
Fibrotic lung disease
Histologic or radiographic evidence suggestive of usual interstitial pneumonia (UIP) or nonspecific interstitial pneumonia (NSIP)
FVC <60% of predicted
Diffusion capacity <39% of predicted (UIP) or <35% of predicted (NSIP)
Drop in FVC by ≥10% or diffusion capacity by ≥15% over a 6-month period
Drop in S_aO_2 on pulse oximetry by <88% on 6-minute walk test
High-resolution CT imaging with honeycombing (fibrosis score >2)
Pulmonary hypertension
Cystic fibrosis
FEV_1 <30% of predicted
P_aO_2 <55 mmHg
P_aCO_2 >50 mmHg
Exacerbations requiring intensive care unit stay
Increasing frequent of pulmonary exacerbations requiring antibiotic therapy
Recurrent and/or refractory pneumothorax
Recurrent hemoptysis not controlled by bronchial artery embolization
Pulmonary hypertension
Progressive weight loss, body mass index <18 kg/m^2
Idiopathic pulmonary arterial hypertension
Low or declining 6-minute walk test at <380
Maximum oxygen intake <10.4 mL/min/kg
World Health Organization functional stage III or IV on maximal medical therapy
Cardiac index <2 $L/min/m^2$
Right atrial pressure >15 mmHg
Failure of intravenous epoprostenol therapy or equivalent

BODE, body-mass index, airflow obstruction, dyspnea, and exercise; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.

Table 3 Absolute contraindications for lung transplantation
Malignancy in the last 2 years except for cutaneous squamous and basal cell tumors, 5-year disease-free interval is prudent
Dysfunction of another major organ system (heart, liver, or kidney) that is not amenable to treatment
Noncurable xtrapulmonary infection (active viral hepatitis B, hepatitis C, human immunodeficiency virus)
Significant chest wall/spinal deformity
Nonadherence and/or inability to follow through with medical therapy or office follow-up
Untreatable psychiatric or psychologic condition(s) associated with the inability to cooperate or comply with medical therapy
Lack of dependable social support system
Substance addiction (alcohol, tobacco, or narcotics) within the last 6 months

Table 4 Relative contraindications for lung transplantation
Age older than 65 years
Critical or unstable clinical condition
Severely limited functional status with poor rehabilitation potential
Colonization with highly resistant or highly virulent bacteria, fungi, or mycobacteria
Severe obesity defined as a body mass index (BMI) exceeding 30 kg/m^2
Severe or symptomatic osteoporosis
Mechanical ventilation
Other medical conditions that have not resulted in end-stage organ damage, such as diabetes mellitus, systemic hypertension, peptic ulcer disease, or gastroesophageal reflux should be optimally treated before transplantation

cenocepacia and extreme caution used in offering transplant in the presence of *Mycobacterium abscessus*. Relative contraindications are determined by the individual centers with updated recommendations under development to be soon available.

Clinical outcomes

Survival after LTx in adult patients has slowly improved over the last 30 years (2). One contributing factor is the increasing number of bilateral lung transplants being performed, especially in the younger patient population (*Figure 3*). The improvement in survival has improved in a stepwise fashion as outlined in *Figure 4*.

Innovations

Hardy *et al.* were clearly innovative in 1963 when they performed the first lung transplant. Novel discoveries continue to influence the outcomes of patients with advanced lung disease regarding LTx. The use of extracorporeal support has made an immediate impact as it is commonplace for patients to be bridged to LTx with extracorporeal membrane oxygenation (ECMO) (6-16), but ECMO remains to be a relative contraindication in the current published guidelines, thus the need for an update. The use of ECMO as a means to bridge was recently reported with similar outcomes as lung retransplantation (6). A major innovation with the advent of normothermic ex vivo lung perfusion by the group at the University of Toronto has resulted in the successful transplantation of donor lungs that would have been previously discarded (17,18). This technology uses extracorporeal means to support donor organs. More recently, induction immunosuppression was shown to have a significantly positive effect on survival (19). Discoveries continue to include modifications of currently available treatments as best practice still continues to evolve in LTx.

Conclusions

Based on the recent advancements, the future is very bright in the care of patients with advanced lung disease who require LTx. Despite recent novel discoveries and innovations, further work is needed to improve and enhance not only the current technologies and treatments, but how

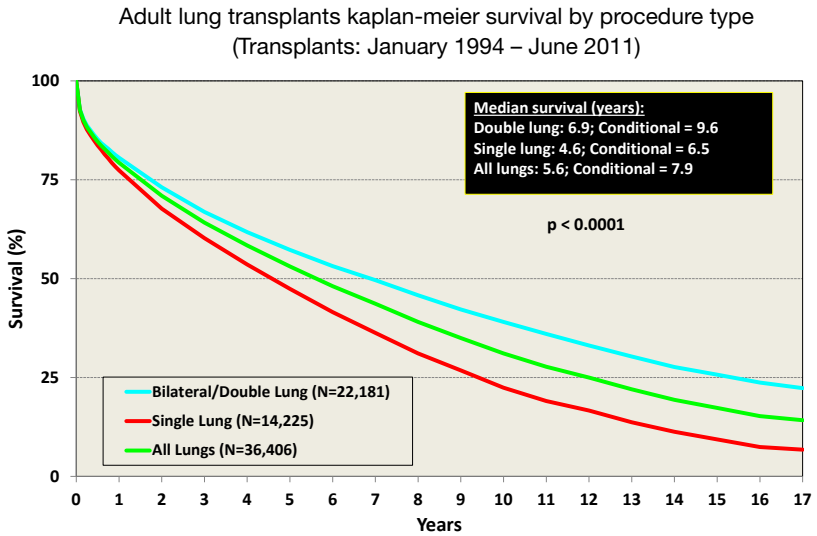

Figure 3 Adult lung transplants Kaplan-Meier survival by procedure type (single or bilateral) from January 1994 to June 2011, modified with permission (2). Survival was calculated using the Kaplan-Meier method, which incorporates information from all transplants for whom any follow-up has been provided. Since many patients are still alive and some patients have been lost to follow-up, the survival rates are estimates rather than exact rates because the time of death is not known for all patients. The median survival is the estimated time point at which 50% of all of the recipients have died. The conditional median survival is the estimated time point at which 50% of the recipients who survive to at least 1 year have died. Because the decline in survival is greatest during the first year following transplantation, the conditional survival provides a more realistic expectation of survival time for recipients who survive the early post-transplant period. Survival rates were compared using the log-rank test statistic.

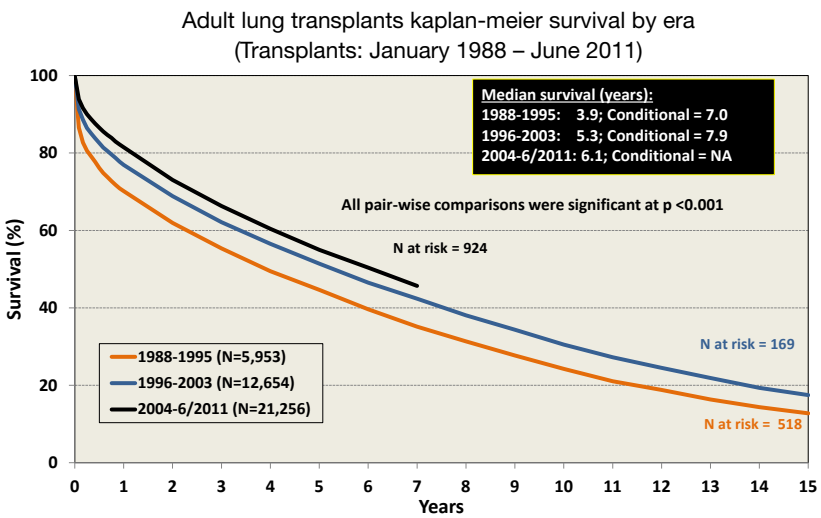

Figure 4 Adult lung transplants Kaplan-Meier survival by era from January 1988 to June 2011, modified with permission (2). Survival was calculated using the Kaplan-Meier method, which incorporates information from all transplants for whom any follow-up has been provided. Since many patients are still alive and some patients have been lost to follow-up, the survival rates are estimates rather than exact rates because the time of death is not known for all patients. The median survival is the estimated time point at which 50% of all of the recipients have died. The conditional median survival is the estimated time point at which 50% of the recipients who survive to at least 1 year have died. Because the decline in survival is greatest during the first year following transplantation, the conditional survival provides a more realistic expectation of survival time for recipients who survive the early post-transplant period. Survival rates were compared using the log-rank test statistic. Adjustments for multiple comparisons were done using Scheffe's method.

we use them and in what clinical situation. Multi-center studies are badly needed in order to even further improve outcomes in LTx.

Acknowledgements

No funding was required to complete this work which was completed at The Ohio State University and Nationwide Children's Hospital.

Footnote

Conflicts of Interest: The authors declare no conflict of interest.

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Cite this article as: Whitson BA, Hayes D Jr. Indications and outcomes in adult lung transplantation. J Thorac Dis 2014;6(8):1018-1023. doi: 10.3978/j.issn.2072-1439.2014.07.04

Pediatric lung transplantation: indications and outcomes

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Abstract: Lung transplantation (LTx) is a treatment option for infants and children with untreatable and otherwise fatal pulmonary diseases. To date, over 1,800 lung transplants have been performed, most frequently in children over the age of five years. The most common indications for transplantation in children overall are cystic fibrosis (CF) and idiopathic pulmonary hypertension (PH). The surfactant protein deficiencies, other interstitial lung diseases (ILDs), and congenital heart disease are important indications among young children and infants. Re-transplantation is an option for selected recipients with chronic allograft rejection. Overall survival following pediatric LTx is similar to that encountered in adult patients, with recent registry data indicating a median survival of 4.9 years. Other outcomes such as the incidence of bronchiolitis obliterans (BO) and the presence of key post-transplant co-morbid conditions are also similar to the experience in adult lung transplant recipients.

Keywords: Lung transplantation (LTx); pediatrics; infants; indications; survival

Submitted Jan 21, 2014. Accepted for publication Apr 18, 2014.
doi: 10.3978/j.issn.2072-1439.2014.04.27
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.04.27

Introduction

Lung transplantation (LTx) is a therapeutic option for children and infants with incurable and end-stage diseases of the lungs or pulmonary vascular system. While LTx in this special age group carries unique challenges, there is ample evidence to suggest that outcomes are similar to those in adults. Pediatric LTx offers the potential for prolonging life expectancy and also improving quality-of- life. The purpose of this paper is to review the most common indications for LTx in pediatric patients and to present available outcomes data for children undergoing this procedure.

The era of LTx began over 50 years ago when Hardy and colleagues performed the first transplant in 1963 in a 58 year-old man with bronchial carcinoma (1). Since that time, significant progress in the field has been made in regards to surgical technique, immunosuppressive regimens, recognition and treatment of allograft rejection, and the development of multidisciplinary and collaborative surgical and medical teams to provide optimal long-term care (2-5). Over 43,000 LTx have been performed in adults with the most common indications being COPD, pulmonary fibrosis and cystic fibrosis (CF) (6).

The first pediatric LTx involved a 16 year-old boy with familial pulmonary fibrosis and was performed in 1987 at the University of Toronto (4). Successful LTx has subsequently been performed in children of all ages, including infants, yet the majority of pediatric cases involve children over the age of 11. The most recent registry data of the International Society for Heart and Lung Transplantation (ISHLT) reports that 1,875 lung transplantations have been performed in pediatric patients, most commonly for a diagnosis of CF (7). There is clear evidence that survival after pediatric LTx has improved in recent years, a trend most reflective of improvement in early survival (8). As the total volume of pediatric transplants is far exceeded by those performed in adults, it is not surprising that the total number of centers providing LTx in children and infants is small. In 2011, only 43 centers reported LTx in children with the majority being located in North America and Europe. In addition, most pediatric centers have very low volumes compared to adult programs, with only one center performing more

Table 1 Most common indication for pediatric lung transplantation. Data adapted from 2012 ISHLT registry report (7)

Age group	Indication for transplant	Total transplants in age group (%)
11-17 years	CF	71
	IPAH	8
	Re-transplant	5
6-10 years	CF	53
	IPAH	9
	BO (non-retransplant)	7
	Retransplant	6
	IPF	6
1-5 years	IPAH	22
	IPF	17
	Pulmonary fibrosis (other)	9
	Retransplant	9
<1 year infants	Surfactant protein b deficiency	17
	Congenital heart disease	17
	IPAH	13

CF, cystic fibrosis; IPAH, idiopathic pulmonary arterial hypertension; BO, bronchiolitis obliterans; IPF, idiopathic pulmonary fibrosis.

than 10 transplants per year. The total number of children undergoing LTx each year has been slightly greater than 100 from 2006-2011.

There are several important anatomical, physiological, psychosocial and epidemiological factors that are indeed unique to LTx in children and infants (2,4,9,10). First, the size of both pediatric lung donor and recipient may present special surgical challenges with regards to size matching and bronchial and vascular anastomoses. The immune systems of children, and infants in particular, are immature and developing and therefore unlike those of adults. It has been suggested that young children may have less risk of acute and chronic allograft rejection and therefore have more tolerance of transplantation (11). There is also evidence that certain infectious issues, particularly seasonal respiratory tract viruses, are of paramount importance in pediatric LTx (12,13). Nutrition, gastroesophageal reflux disease, and risk of aspiration may all have direct influence on morbidity and survival in children (14). Another important factor in successful LTx in pediatric patients is appropriate parental support to provide for the very complex post-transplant care. Unreliable psychosocial circumstances can in fact be a major obstacle to long term success (15). Adolescents in particular may struggle with adherence to prescribed therapies as the mature and gain more independence. Taken together, these special considerations in pediatric LTx are important factors to consider in evaluating a potential patient for transplant candidacy.

Indications for pediatric lung transplantation (LTx)

CF is the most common indication for LTx in pediatric patients overall and was the primary diagnosis in 1,063 of 1,875 (57%) children in the IHSLT registry (5). Idiopathic pulmonary arterial hypertension (IPAH) is the second most-common indication for LTx, and 164 cases (9%) have been reported. Other less common but important indications for pediatric LTx include: idiopathic pulmonary fibrosis (IPF), surfactant protein deficiencies and other diseases now more uniformly classified as childhood interstitial lung diseases (chILD), congenital heart disease, and re-transplantation.

There is substantial variability in the indication for LTx among sub-groups of pediatric patients divided by age. For instance, in older children and adolescents aged 11-17 years, CF is the indication for LTx in 70% of cases. However CF becomes less predominant in younger aged patients, representing 53% of LTx in children 6-10 years of age and less than 5% in young children under age 5. The most common indication for LTx among children age 1-5 and 6-10 years of age is IPAH. Congenital heart disease, the chILD syndromes including surfactant protein B deficiency (SP-B), and IPAH are the most common indication for infant LTx. The most common indications for pediatric LTx categorized by age group are demonstrated in *Table 1*.

Cystic fibrosis (CF)

CF is the most common fatal genetic disease affecting Caucasian populations worldwide. This disease is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a chloride channel responsible for ion transport across epithelial cells lining the respiratory tract. Abnormal CFTR results in dehydration of the airways, thick mucus, and poor mucociliary clearance. This causes a cycle of obstruction of the airways by viscous mucus, chronic airway infection, and chronic lung and systemic inflammation. Chronic respiratory failure from CF lung disease is the most

common cause of death (16,17). The hallmarks of treatment are airway clearance via chest physiotherapy, aerosolized medications that can help rehydrate and reduce mucus viscosity, anti-inflammatory therapies, aggressive treatment of both chronic and acute on chronic infections, and optimization of key CF comorbidities such as malnutrition and diabetes mellitus (16,18). More recently developed genotype-specific therapies may help correct the underlying CFTR defect which causes CF (19). Survival for CF has improved dramatically over the last several decades with the most recent median survival exceeding 41 years (20). However, despite improvements in treatment and improved survival, LTx remains an important treatment option for advanced CF lung disease in childhood and adolescence. CF is the most common reason for transplant in pediatrics and CF is the third most common indication among adults.

Idiopathic pulmonary arterial hypertension (IPAH)

IPAH is the second most-common indication for LTx in pediatric patients overall, and is the most common indication among children aged 1-5 years. Pulmonary hypertension (PH), in general, is defined by a mean pulmonary artery pressure at rest greater than 25 mm Hg and a pulmonary vascular greater than 3 Woods after three months of age (21). Recent classification strategies by the World Health Organization and the Pediatric Task Force of the 5th World Symposium convening in Nice, France [2013] have further grouped patients with PH into several main categories by main mechanism of elevation in pulmonary artery pressure (22). A detailed description of the classification schema of PH is beyond the scope of this paper, but Group 1 (pulmonary arterial hypertension) diseases which include IPAH, heritable PAH, and PAH associated with congenital heart diseases are the most frequently encountered entities causing end-stage cardiopulmonary disease in pediatric patients.

The natural history of untreated IPAH is one of rapid clinical deterioration and frequent death, often within three years of initial diagnosis. The progression of children with PH may be more rapid than in adult patients (23-25). However, in recent years the development of more effective pulmonary vasodilator medications, in particular the prostacyclin based therapies, has demonstrated clear improvements in survival (21). Despite the benefits of IPAH medications, the ultimate outcome in most pediatric patients is death and therefore LTx remains an important and viable treatment strategy (26). Current guidelines in adults would suggest referral for LTx when patients reach New York Heart Association functional classification of level III to IV, meaning patients who are symptomatic with exertion or at rest. The applicability of these subjective categories in young children may be of limited utility, however there is evidence that children with supra-systemic right heart pressures and those who experienced hemoptysis were at increased risk for death on the waitlist (26). This would suggest that children with IPAH and these poor prognosticating features should be listed early for transplantation.

Interstitial lung disease (ILD) and surfactant protein deficiencies

It has been well-recognized that ILD in pediatric patients differs significantly from that in adults (27). The chILD syndromes have been described as a heterogeneous group of disorders affecting children less than 2 years old with respiratory signs and symptoms (most frequently tachypnea), impairment in gas exchange (hypoxemia) and evidence of diffuse parenchymal lung disease on chest imaging. The American Thoracic Society has recently published clinical guidelines for the diagnosis and management of these patients (28). The chILD syndromes can be sub-divided as those syndromes affecting infants and those not specific to infancy.

The surfactant protein deficiencies are quite rare diseases but are the most common indication among the chILD diseases for LTx in infancy. There have been four surfactant protein deficiency syndromes described including SP-B, surfactant protein C deficiency (SP-C), adenosine triphosphate binding cassette protein member A3 (ABCA3), and thyroid transcription factor (*NKX2.1* gene) (29-31). The presentation of the surfactant deficiencies may vary from severe hypoxemic respiratory failure in the newborn period (32) (typical of SP-B) to a more insidious development of tachypnea, hypoxemia and diffuse interstitial changes on chest imaging later in infancy (more typical of SP-C) (33). Diagnosis of these syndromes can be achieved through genetic sequencing technology (28). Perhaps the most important (and most aggressive) surfactant protein deficiency is SP-B, which is recognized as a universally fatal disease and LTx is considered the only viable treatment option (34).

Other important chILD syndromes that may lead to LTx in infants include disorders of lung development such as alveolar capillary dysplasia with misalignment of pulmonary veins (a disease affecting infants in the newborn period that is believed to be uniformly fatal) and growth abnormalities

such as neonatal chronic lung disease (bronchopulmonary dysplasia) (35).

BO and re-transplantation

BO refers to obstructive lung disease resulting from bronchiolar inflammation and is described pathologically by circumferential peribronchial fibrosis that can constrict or completely obliterate the lumen of the bronchiole (36). BO can be caused by infectious or non-infectious insults to the airways which trigger the process of inflammation and fibrosis. Post-infectious BO in children is frequently associated with severe viral (adenovirus) or mycoplasma infections (37). Non-infectious BO can occur in children as a consequence of autoimmune diseases, inhalational injuries, and Stevens-Johnson syndrome among others. However, a very important cause of BO is post-transplant in nature. BO can occur as a consequence of pediatric bone marrow transplantation (38,39). Any of these specific etiologies of BO can ultimately manifest in respiratory failure and be an appropriate indication for LTx in children.

The most common group of patients with BO undergoing consideration for LTx is in fact primary lung recipients who develop chronic allograft dysfunction over time. BO remains the major obstacle to long term success in LTx recipients and current treatment options are limited (40). Therefore BO following initial LTx remains an important indication for consideration of re-transplantation. Pediatric patients may be given special consideration for re-transplantation, as achieving an expected graft survival and therefore "good outcome" defined by some standards may not allow a child to reach adulthood. The indications for pediatric lung re-transplant can generally be classified as those patients with chronic allograft dysfunction with BO versus those without BO who suffer graft failure from other causes. A total of 118 pediatric lung re-transplants have been reported, and available data suggests this procedure is most beneficial in patients with chronic graft failure occurring greater than 1 year post-initial transplant (28,41).

Outcomes

Although the most common indications for LTx in pediatric patients differ from those of adults with end-stage lung disease, the available data on outcomes suggest that the success of LTx is quite similar. While survival is certainly the paramount outcome measure for LTx recipients of all ages, other variables such as the incidence of graft rejection, the frequency of key comorbid conditions, the need for re-transplantation, and overall quality of life and functional status are also clinically important.

Survival data

The annual IHSLT Registry report is the most comprehensive database of thoracic LTx performed worldwide (28). Participation in this registry is voluntary but it is believed that this data encompasses the vast majority of pediatric LTx performed each year. In 2011, a total of 43 centers performed LTx in pediatric patients with the vast majority of these centers located in Europe (n=20) and North America (n=18). The 2013 ISHLT registry data of pediatric LTx performed between 1990 and 2011 reports a median survival of 4.9 years for pediatric patients. This observed survival is statistically similar to that of adult LTx recipients (4.9 versus 5.4 years, P=0.3459, *Figure 1*) Like in adults, there has been a clear improvement in survival when comparing era of transplant, with median survival of 3.3 years among those transplanted between 1988-1999 versus median survival of 5.8 years in those transplanted in the modern era of 2000-2011 ($P \leq 0.001$). Pediatric patients with CF have similar survival to those without CF, with median survival of 4.7 years in both groups (*Figure 2*). While it appears children age 6-10 years may have improved early survival, there is no clear difference in overall long term survival (*Figure 3*). The 2012 US Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients (OPTN/STRS) data analysis of pediatric LTx performed in the US in 2007-2008 reported post-transplant survival of 96.3% at 30 days, 87% at one year, 60.1% at 3 years, and 49% at 5 years (42).

The most common cause of death in the first 30 days following pediatric LTx is graft failure which accounts for approximately 30% of early mortality (7). Non-CMV infection and graft failure are the most common causes of death from one month to one year post transplant, and account for over 50% of mortality in this time period. Bronchiolitis obliterans syndrome (BOS), like in adults, is the most common cause of death after the first year following pediatric LTx, and represents 40% of deaths at both 1-3 and 3-5 years post-transplant. BOS is responsible for 47% of deaths after 5 years (28). Thus BOS remains the biggest obstacle to long-term survival in both pediatric and adult LTx recipients. This data is consistent with that presented in the OPTN/STRS database (42).

The available data on re-transplants in pediatric

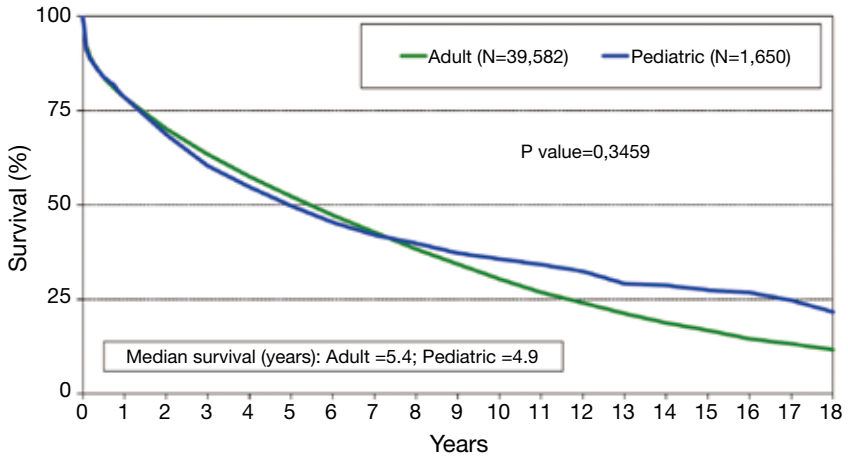

Figure 1 Median survival in pediatric lung transplant recipients compared to adults (7).

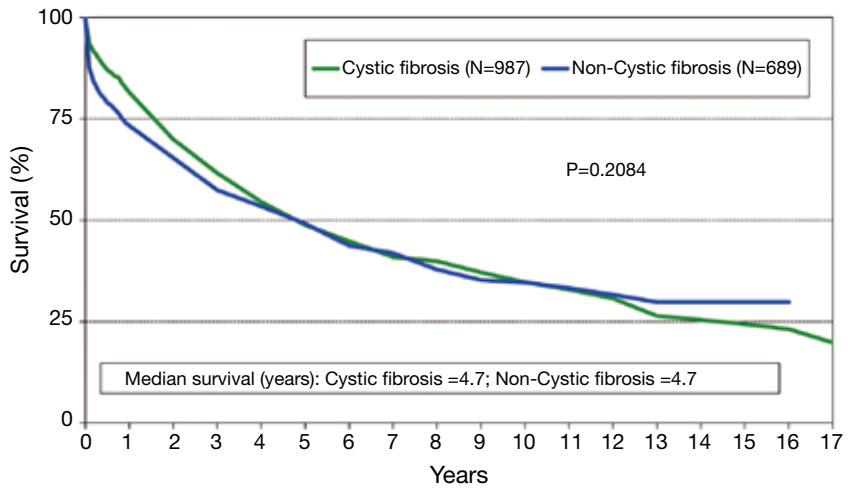

Figure 2 Median survival in pediatric lung transplant recipients with CF versus non-CF (7).

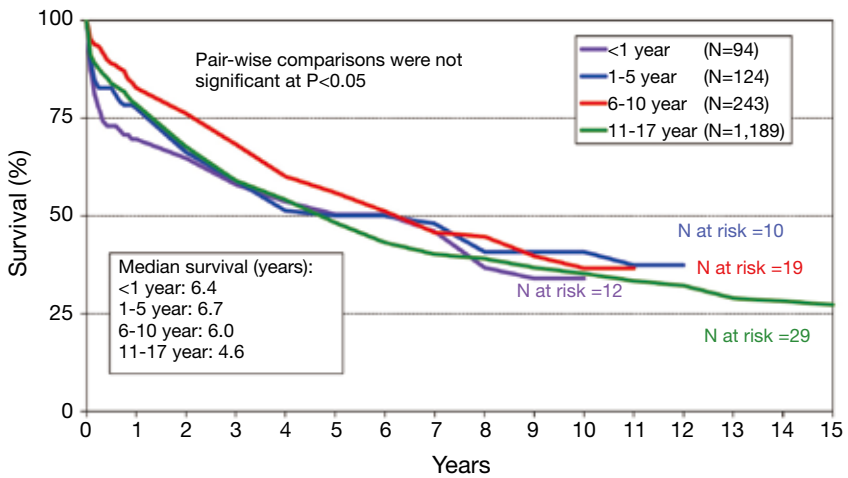

Figure 3 Median survival in pediatric lung transplant recipients by specific age group (7).

patients suggest that outcomes are worse compared to the initial transplant. There were 118 pediatric re-transplants performed between 1994 and 2012, and the approximately 23% of these procedures were performed within 12 months of the original. Three year survival among re-transplants is significantly lower than that of primary transplants (58% *vs.* 45%, P=0.026.) There was no significant difference between indications for re-transplant when comparing BOS to non-BOS related cases (28). It appears that the best outcomes for pediatric lung re-transplant are achieved in patients that are further than one year removed from initial transplant and are not ventilator dependent (41).

There are multiple studies that highlight survival characteristics and special considerations among specific groups of pediatric LTx recipients. A controversial study regarding the benefit of LTx for children with CF, published by Liou and colleagues, analyzed data from the CF Foundation and United Network for Organ Sharing databases between the years 1992 and 2002 (43). This group concluded that few children with CF achieved an overall survival benefit from LTx. Since that time, a large analysis of adult data from 2005-2009 (the lung allocation score era) demonstrated a strong improvement in adults with CF undergoing LTx (44). Many pediatric LTx centers and leading experts in the field have cited several reasons why the data from Liou at al may not be applicable to individual children with CF in the current era of LTx, citing the transition to the current lung allocation system in the US as well as controversies regarding the statistical analysis and cohort used in the study among others (45,46). There is no clear evidence that the frequency of pediatric LTx for CF has decreased in recent years, although with advances in the care of CF patients it is reasonable to anticipate a future shift towards more transplantations occurring in adulthood as opposed to childhood or adolescence. Over the past decade, it has become clear that CF patients with chronic infection with *Burkholderia cenocepacia* infection are particularly at risk for poor outcomes following LTx, primarily due to infection in the post-transplant period (47,48). Therefore, infection with *B. cenocepacia* is considered a contraindication at most centers.

Among diseases other than CF, there is data to suggest equivalent post-transplant survival. For instance, a retrospective single center review of 26 children undergoing LTx for IPAH showed a median survival of 5.8 years and 1- and 5-year survival of 95% and 61% respectively (26). Likewise, a multicenter retrospective chart review of 31 children undergoing LTx for diffuse lung disease (encompassing the chILD syndromes) showed comparable survival compared to children undergoing LTx for other indications (49).

The most common indications for LTx in infants are SP-B deficiency, congenital heart disease, and IPAH. Successful LTx in infants may be particularly challenging due to factors such as donor availability, size of the donor and recipient, risk of post-transplant respiratory viral infection, and other physiological factors such as aspiration risk. Infant LTx is a very rare procedure performed only at a handful of centers. In 2011 only four infant transplants were performed in the US, a number far below the number of heart transplants performed in this age group (42). An analysis of the UNOS database reported similar overall survival among 80 infants (<1 year of age) compared to older children and adolescents (age 1-18 years). This study also suggested an improved conditional survival for those infants surviving at least 1 year (50). This data suggests a potential protective advantage of the immature immune system of infants, and is corroborated by a previous study demonstrating a decreased incidence of allograft rejection among infants (11).

Outcomes other than survival

There appears to be a similar incidence of key post-transplant comorbid conditions following LTx in both pediatric and adult populations. The most commonly encountered co-morbidities at one year following LTx in pediatrics include hypertension, renal dysfunction, hyperlipidemia, and diabetes mellitus. These same conditions increase in frequency in survivors at 5 years post-transplant (7).

It may be challenging to assess functional status and quality of life in pediatric patients who may not be able to express their feelings adequately, and secondary reports from parents or physicians may be confounded by bias. However, the ISHLT registry did report that more than 80% of pediatric LTx recipients were given favorable assessments of functional status as measured by reported Lansky scores (7).

Summary

Pediatric LTx is a viable treatment option for infants and children with end-stage pulmonary diseases. The most common indications for children are CF and IPAH, while the chILD syndromes and congenital heart disease are the predominant indication for infants. Overall survival after LTx in the pediatric population is similar to the expected

survival in adults. Chronic allograft rejection remains the biggest obstacle to more prolonged survival, and re-transplantation in select patients may be a reasonable treatment option.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Kirkby S, Hayes D Jr. Pediatric lung transplantation: indications and outcomes. J Thorac Dis 2014;6(8):1024-1031. doi: 10.3978/j.issn.2072-1439.2014.04.27

Ex vivo lung perfusion

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Abstract: Lung transplantation (LTx) is an established treatment option for eligible patients with end-stage lung disease. Nevertheless, the imbalance between suitable donor lungs available and the increasing number of patients considered for LTx reflects in considerable waitlist mortality. Among potential alternatives to address this issue, ex vivo lung perfusion (EVLP) has emerged as a modern preservation technique that allows for more accurate lung assessment and also improvement of lung function. Its application in high-risk donor lungs has been successful and resulted in safe expansion of the donor pool. This article will: (I) review the technical details of EVLP; (II) the rationale behind the method; (III) report the worldwide clinical experience with the EVLP, including the Toronto technique and others; (IV) finally, discuss the growing literature on EVLP application for donation after cardiac death (DCD) lungs.

Keywords: Ex vivo lung perfusion (EVLP); lung transplantation; organ preservation

Submitted Apr 16, 2014. Accepted for publication Jul 01, 2014.
doi: 10.3978/j.issn.2072-1439.2014.07.12
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.07.12

The shortage of donor lungs

According to the *Thirtieth Adult Lung and Heart-Lung Transplant Report 2013*, from the Registry of the International Society for Heart and Lung Transplantation, lung transplantation (LTx) is a therapy that is being performed worldwide, with numbers increasing every year (1). In 2011, 3,640 LTxs were reported compared to only 1,712 annual cases a decade ago. As the outcomes tend to improve, an increasing number of patients with end-stage lung disease are being considered for LTx. Nevertheless, the amount of lungs suitable for transplantation has not followed this trend and this equation generates considerable waitlist mortality (15.4 per 100 wait-list years in the US form 2010 to 2012) (2).

Donor lungs are subjected to several injurious mechanisms during the brain death/organ donation process (such as ventilator-acquired pneumonia, neurogenic and hydrostatic pulmonary edema, barotrauma). Thus, it is not surprising that the majority of donor lungs are not utilized for transplantation (39% Eurotransplant 2012, 78% SRTR in the US 2012).

Strategies for lung donor pool expansion

Expansion of the donor pool has been attempted by extending the donor selection conventional criteria, by use of donation after cardiac death (DCD) and, lastly, with the implementation of ex vivo lung perfusion (EVLP). The ideal donor corresponds to a <55 year-old with <20 pack-year smoking history, no chest trauma, clear chest X-ray, P/F >300 and absence of purulent secretions and organisms on gram stain of respiratory samples. This scenario is known to correspond to less than half of the donors utilized for transplantation (3). Several studies addressing the use of extended criteria donors have been published and, more recently, a review study summarized the findings of 10 studies ranging from 1993 to 2010, bringing the best evidence up to date (4). Although no clear differences in mid or long-term survival were observed, 4 of these studies revealed worse early outcomes (such as 30- and 90-day mortality, ICU and hospital stay and gas exchange at ICU arrival). Recently, the Hannover group has shown an interesting algorithm proposing allocation of extended criteria donor lungs to lower-risk recipients. Results were

encouraging and deserve further analysis (5).

Although the first successful LTx was performed from DCD, the concept of using controlled DCD lungs has been clinically revisited by D'Alessandro *et al.*, in 1995 (6). Series of studies have followed, reporting an increasing international experience and highlighting the potential of DCD to partially address the shortage of donor lungs (7-13). Nevertheless, caution is still observed in the transplant community as there are a series of specific injuries that the DCD lung is prone to, specially during the interval from withdrawal of life sustaining therapies to pulmonary artery (PA) flush. Another potential source of lungs comes from the use of uncontrolled DCDs (Maastricht categories I and II). The group of Madrid has explored this peculiar pool, reporting the experience with 29 cases. Ninety-day and 1-year mortality were 22% and 32% respectively, with higher rates of primary graft dysfunction (PGD) 2-3 than expected (14).

The use of lungs from smoker donors has been recently studied in a large registry database including 1,295 transplants (510 with smoking history) from UK. Despite presenting worse 3-year survival, the use of lungs from donors with a positive smoking history was shown to provide a survival benefit for patients with interstitial lung disease listed for transplantation (15). Several recent studies followed and supported the use of such donors (16-18). Nevertheless, caution was raised in the analysis of the UNOS database including 3,704 single-lung transplants from 2005 to 2011. In this modality of transplant, recipients from donors with an active smoking history, but not those from donors that quit smoking, were associated with increased mortality (19).

Lastly, clinical EVLP was shown to safely increase the donor pool by preserving high-risk donor lungs with similar outcomes to standard criteria donor lungs (20). This review will focus on technical aspects of EVLP, its recent clinical experience and pre-clinical application in DCD.

EX vivo lung perfusion

Perfusion of whole organs was initially envisioned by Alexis Carrel and Charles Lindbergh. In the 30's, they performed several experiments with organs such as heart, kidney, thyroid, ovary, adrenal glands and spleen (21). Up to the 90's, experiments with lung perfusion were viewed as a reliable method to study pulmonary physiology. The first clinical application was described by Steen and coworkers at University Hospital of Lund. In 2001, they described the utilization of EVLP to assess the lungs of a 54-year-old who suffered a myocardial infarction while admitted to the intensive care unit. Lungs were topically cooled with perfadex and procured after 190 minutes of cardiopulmonary resuscitation cessation. EVLP was performed for 65 minutes and a successful right single lung transplant was performed (22). The same group further expanded the application of short-period EVLP to lungs initially rejected for transplantation. A total of 6 sets of donor lungs were perfused from 61 to 121 minutes, rendering six successful double lung transplants (23). The Toronto group mastered the technique and introduced the concept of extended EVLP, focusing not only on reassessment but also on providing a platform for treatment delivery in the normothermic state (24,25).

EVLP—the Toronto technique

The foundations of our current technique for clinical EVLP are: (I) gradual rewarming up to normothermia; (II) gradual increase in vascular flow as the lungs are rewarmed, targeting 40% of the donor predicted cardiac output; (III) protective lung ventilation; (IV) acellular perfusate with increased colloid osmotic pressure.

The indications for EVLP are listed in *Table 1*. Once at the transplant center, the lungs are dissected on the back table. The left atrial (LA) cuff is trimmed and sewn to a dedicated cannula with two 4-0 polypropylene running sutures (*Figure 1*). If adequate length on the PA is available, the PA cannula can be simply inserted proximal to its bifurcation and secured with two heavy silk ties (*Figure 2*). In cases of short main PA—usually in concomitant heart procurement—a cuffed PA cannula can be sewn with two 5-0 polypropylene running sutures, similarly to the atrium. With the trachea clamped at the level of the carina, the staple line is opened and a conventional endotracheal tube is inserted and secured with two heavy silk ties (*Figure 3*). A second retrograde flush with 1L of Perfadex is performed. The inflated lungs are then taken to the EVLP dome and ready to be connected to the circuit (*Figure 4*). If one of the lungs is judged too damaged for clinical EVLP (e.g., due to pneumonia), the contralateral lung can be perfused alone. Care should be taken to keep adequate arterial and atrial cuffs and a long trachea/bronchus at the moment of division.

The EVLP circuit

A dedicated circuit composed of a centrifugal pump, a

Table 1 Current indications for EVLP for both brain death donors and donors after cardiac death (20,26)

- Best PaO_2/FiO_2 <300 mmHg
- Signs of pulmonary edema either on chest X-ray or physical examination at the donor site
- Poor lung compliance during examination at procurement operation
- High-risk history, such as >10 units of blood transfusion or questionable history of aspiration
- DCDs with >60 min interval from withdrawal life support to cardiac arrest interval

EVLP, ex vivo lung perfusion; DCD, donation after cardiac death.

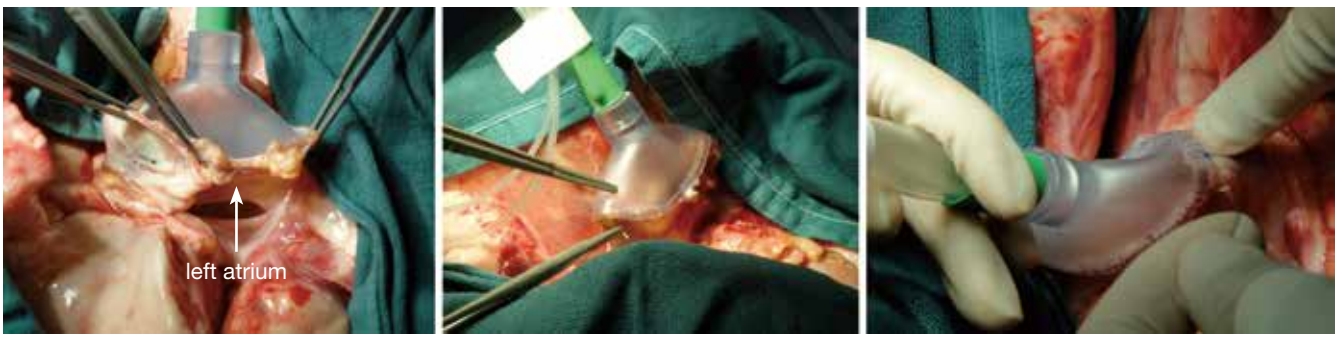

Figure 1 Preparation of the left atrium.

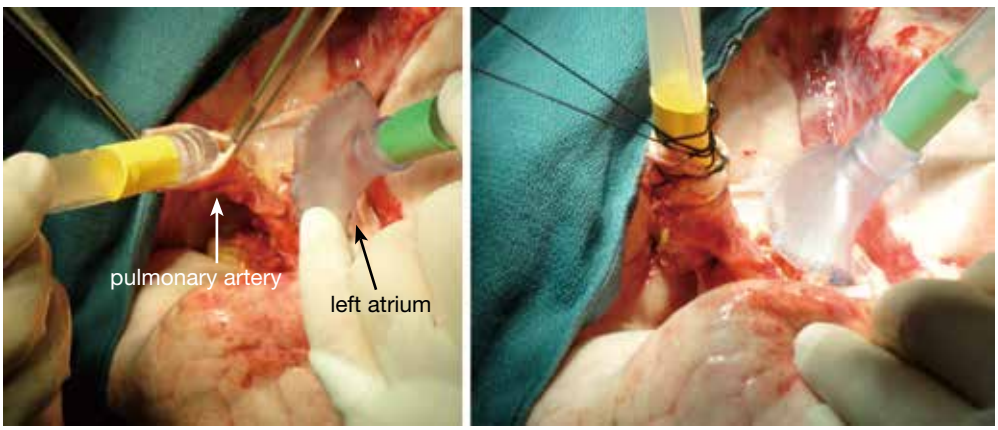

Figure 2 Preparation of the pulmonary artery.

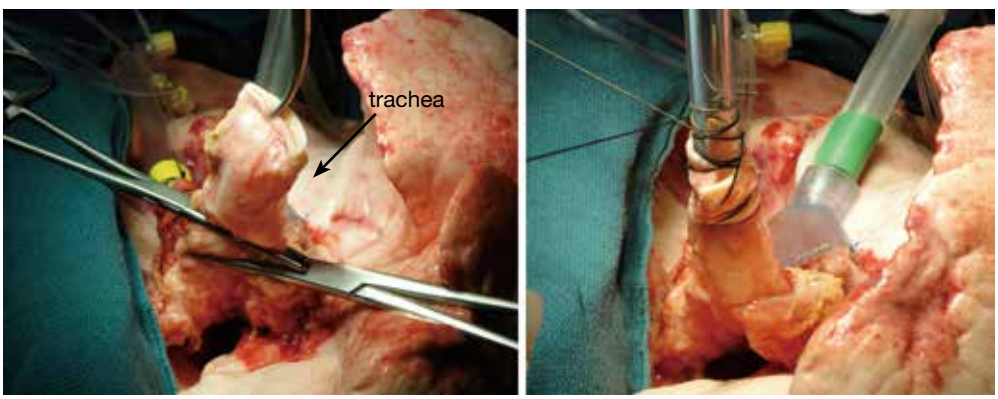

Figure 3 Preparation of the trachea.

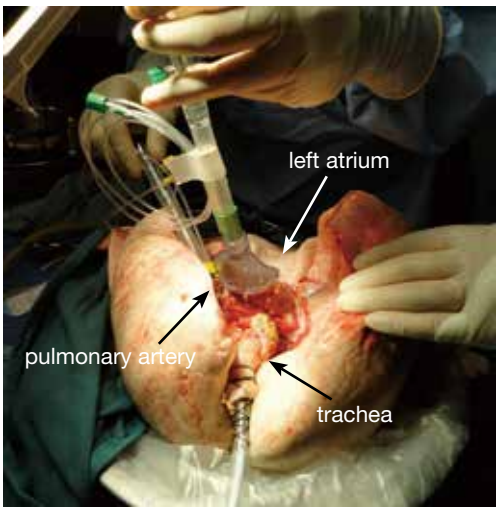

Figure 4 Lungs after cannulation.

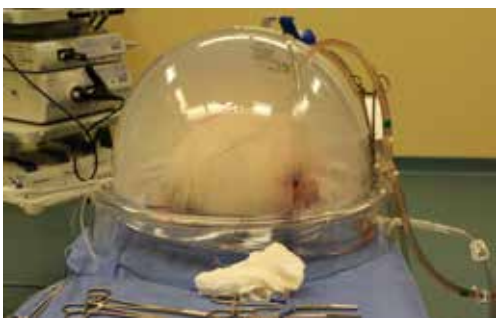

Figure 5 Lungs being ventilated and perfused on steady-state.

leukocyte filter, a hollow-fiber oxygenator heat exchanger and a hardshell reservoir is currently used. It is primed with 2.0 L of Steen solution (XVIVO, Vitrolife), 500 mg methylprednisolone (Solu-medrol; Sandoz Canada, Boucherville, Canada), 3,000 IU of unfractionated heparin (Organon, Canada) and antibiotic (500 mg imipenem/cilastatin, Primaxin; Merck, Whitehouse Station, NJ).

Initiation and steady state

Once on the EVLP dome, a cotton sponge is positioned beneath the lung block to prevent excessive sliding. Antegrade flow is commenced through the PA cannula, which is attached to the circuit once appropriate deairing is achieved. The LA cannula is then deaired and connected to the circuit. The outflow clamp is now removed. Our target perfusion flow consists of 40% of the donor predicted cardiac output. Following our principles of gradual rewarming and stepwise increase in vascular flow, the procedure is then initiated with lungs on room temperature and perfusion with 10% of the calculated target flow. At 10 minutes, the flow is raised to 20% of predicted and the temperature is set to 30 ℃. At subsequent 10-minute time points (20, 30, 40 and 50 minutes), the flow is increased to 30%, 50%, 80% and finally 100% of target, respectively. Furthermore, the temperature is set to 37 ℃ at 20 minutes and ventilation is initiated (7 mL/kg, PEEP 5 cm H_2O and 7 cycles/min) when the temperature reaches 33 ℃. Once the lungs are being ventilated, the gas mixture (86% N_2, 8% CO_2 and 6% O_2) is turned on at a sweep of 1 L/min. The target of a post-membrane pCO_2 between 35-40 mmHg is achieved by titrating the sweep gas. Lastly, the left atrial pressure should be carefully maintained in the 3-5 mmHg range by adjusting the level of the reservoir. Once the lungs are normothermic, ventilated and target flow is achieved, recruitment maneuvers are performed up to 25 cm H_2O. The lungs have now reached the steady state (*Figure 5*). Steen solution is exchanged from the circuit hourly, 500 mL in the first hour followed by 250 mL thereafter.

Assessment mode

Assessment is performed hourly. Ventilation parameters are set to 10 mL/kg tidal volume, 10 breaths per minute and FiO_2 1.0 for five minutes. PA pressure, LA pressure, peak airway pressure, plateau pressure, dynamic and static compliance are recorded. Perfusate gas analysis is done in samples taken from the venous and arterial sides. At 1 hour of EVLP and then every two hours, a lung X-ray is routinely performed. Criteria for lung acceptance or declination for transplantation after EVLP are displayed in *Table 2*. One should notice that the acellular nature of the Steen solution makes perfusate pO_2 a later marker of lung injury. As demonstrated by Yeung and coworkers, compliance and peak airway pressure deterioration are observed before changes in perfusate pO_2 (27). Pulmonary recruitment is performed every 30 minutes after each assessment by increasing the tidal volume with subsequent inspiratory hold maneuvers up to 25 cm H_2O for ten seconds.

Termination of perfusion

Our clinical protocol includes EVLP for four to six hours. Frequently, it is possible to make the decision at three hours (3 assessments, 2 lung X-rays) and send for the recipient. By

Table 2 Acceptance and exclusion criteria after 4-6 hours of clinical EVLP (20,26)
Acceptance criteria after EVLP
P/F ratio >400 mmHg
Stable or improving pulmonary artery pressure
Stable or improving airway pressure
Stable or improving pulmonary compliance
Exclusion criteria after EVLP
P/F ratio <400 mmHg
Greater than 15% deterioration on pulmonary artery pressure
Greater than 15% deterioration on airway pressure/compliance

EVLP, ex vivo lung perfusion.

the fourth hour the recipient will be relatively ready for skin incision. Nevertheless, if no clear decision can be made at this time point, perfusion can be extended for up to 6 hours.

Once decision is made to terminate perfusion, lungs are ventilated with 0.5 FiO_2 and cooled to 15 ℃. The inflow and outflow cannulae are clamped and cut. The endotracheal tube is clamped as well with special attention to maintain the lungs inflated. A last antegrade flush is performed with 500 mL of Steen solution. The vascular cannulae are removed and the trachea is stapled just below the endotracheal tube. Topical cooling with Perfadex and ice follows the same steps of conventional preservation and the lungs are taken to the recipient OR inside a cooler.

Worldwide experience with clinical EVLP

The Toronto technique

The Toronto Lung Transplant Program conducted a nonrandomized clinical trial to assess the feasibility of EVLP selecting high-risk donor lungs for this modality of preservation (20). A total of 23 donor lungs were submitted to EVLP with 20 being ultimately transplanted (15 bilateral and 5 unilateral lung transplants). The primary end-point of the study (PGD grade 2 or 3 at 72 hours) was recorded in 15% of the EVLP group and 30% of the contemporary no EVLP controls (116 cases), with no significant difference. Secondary end-points such as PGD 2 or 3 at ICU arrival, 24 and 48 hours; ECLS requirement; days on mechanical ventilation; ICU stay; hospital stay and 30-day mortality were also comparable between groups. This experience was recently updated with a total of 50 lung transplants from 58 EVLPs (86% yield) (26). In the study period, from September 2008 to December 2011, 253 lung transplants were performed with conventional preservation lungs. PGD 3 at 72 hours was recorded in 2% EVLP *vs.* 8.5% control (P=0.14). Again, time on mechanical ventilation, ECLS requirement, ICU stay, hospital stay and 30-day mortality were not different. Furthermore, similar 1-year survivals were observed: 87% for EVLP group *vs.* 86% for the standard group.

In 2012, the group from Vienna reported their experience with 13 clinical EVLPs which rendered nine double-lung transplants (69% yield) (28). Early outcomes such as days on mechanical ventilation, ICU stay, hospital stay and 30-day mortality were comparable to 119 contemporary conventional preservation transplants. Of notice, some modifications from the Toronto technique were implemented: (I) decision was made at two hours of perfusion if physiologic parameters were met; (II) recruitment maneuvers were performed 10 minutes before assessments (as opposed to 30 minutes); (III) lungs were ventilated for 15 minutes on 1.0 FiO_2 for each assessment (as opposed to five minutes). Interestingly, all the four declined cases developed massive pulmonary edema and were recovered from donors with trauma history.

The groups from Toronto, Vienna and Paris presented their clinical EVLP experience at the 2013 ISHLT meeting (29). A total of 125 clinical EVLPs were performed with an 82.5% yield. Similarly to previous uni-institutional reports, the incidence of PGD3 at 72 hours was 5% and the 12-month mortality was 12%.

In 2012, the Harefield Hospital (UK) reported six double lung transplants generated from 13 EVLPs (yield 46%) (30). Although the median requirement of mechanical ventilation post-transplant was greater than seven days, all patients ultimately left the hospital and were alive at three months. The Toronto technique was implemented with some

modifications, such as shorter perfusion times (average 2 hours) and no interval lung X-ray in 50% of the accepted cases.

The group of Torino described nine EVLPs rendering seven lung transplants (yield 78%) (31). These cases corresponded to 30% of their LTx activity and illustrated the impact of EVLP on lower volume centers.

In the Newcastle experience with 6 lung transplants from 18 EVLPs (yield 33%), all patients survived to hospital discharge (32). Furthermore, this report pointed to a possible benefit of EVLP: bacterial loads in bronchoalveolar lavages at the end of EVLP were significantly lower than on samples taken at its initiation. Authors also reported that, despite decrease in the bacterial loads there was an increase in the load of *Candida sp.* in two of their first three cases. After this observation, Amphotericin B was routinely added to the perfusate. Further studies are required to better elucidate the role of anti-fungal therapy in EVLP.

The NOVEL Lung trial is an FDA mandated multicenter clinical trial (NOVEL Lung Trial) studying EVLP for marginal donors. The initial report included 31 patients that received EVLP lungs. Early outcomes such as PGD, length on mechanical ventilation, ICU stay, hospital stay and 30-day mortality were similar to 31 non-EVLP controls (33). At the 2014 ISHLT meeting, the trial results were updated to 76 EVLPs rendering 42 lung transplants (55% conversion rate) (34). In comparison with 42 contemporary controls, early outcomes and 1-year survival were not different.

The Lund technique

The main differences from the Toronto technique reside in the open left atrium, the use of Steen solution mixed with red blood cells and the perfusion at flows correspondent to 100% of the donor predicted cardiac output (35).

Following the successful case in 2001 (22), Steen and coworkers reported the use of EVLP for the evaluation of 9 donors lungs rejected for transplantation (23). Ultimately, 6 double lung transplants were performed, representing 35% of the lung transplant activity for the study period. Although two patients died early on the post-transplant course (one 63-year-old COPD male died at 95 days due to sepsis and multi-organ dysfunction; one 64-year-old COPD female died at 9 months due to rejection); the remaining four were followed for almost 2 years and presented good lung function.

The group from University of Gothenburg has reported their outcomes with 11 EVLPs over an 18-month period (36). A total of eight double and three single LTxs were performed and, although hospital stays were similar, the time on mechanical ventilation and ICU length of stay were longer in the EVLP group compared to conventional transplants. Nevertheless, there was no hospital mortality in the EVLP group.

Reflecting the widepread utilization of EVLP by LTx programs throughout the world, the group from Copenhagen recently reported the Danish experience with 7 EVLP lung transplants (37). This number corresponded to 21% of the yearly activity and the outcomes were favorable despite one death at 104 days post-transplant due to *Mycobacterium abscessus* infection.

The portable *ex vivo* technique

This system is capable of transportation in addition to ventilation/perfusion. Similarly to the Lund technique, the left atrium is kept open and red blood cells are added to the perfusate (a modified low-potassium dextran solution). The perfusion flow is set to 2.5 L/min (38).

A pilot study assessing preservation and transportation of conventional criteria donor lungs was published in 2012 by the programs of Hannover and Madrid (38). A total of 12 patients were transplanted, with perfusion times ranging from 188 to 622 minutes. All cases were bilateral LTxs and there was no PGD 3 at 72 hours. Currently there is an ongoing multicentre clinical trial assessing the feasibility and potential benefits of this strategy for extended criteria donor lungs.

EVLP as a platform for assessment and treatment of DCD lungs

There is a growing body of research focusing on the application of EVLP to assess and repair DCD lungs. The low clinical utilization rates of these lungs are likely driven by the different injuries (such as warm ischemia, hypoxia, hypotension and aspiration) that they are prone when compared to neurological determination of death donors (39). The potential of EVLP to further refine DCD lung selection is well illustrated by the pre-clinical report of Sanchez *et al.*, showing that improved endothelial function reflected in better EVLP physiological performance in porcine lungs treated with pre-arrest heparinization (40).

Furthermore, the use of EVLP as a platform to deliver

different medications has been tested and proved to be beneficial in most reports. Nakajima and coworkers have added nitroglycerin and dibutyryl cyclic adenosine monophosphate to Steen solution during EVLP of lungs submitted to 4 hours of warm ischemia (41). After single LTx, EVLP lungs had better function, lower histological signs of acute lung injury and improved microvascular patency compared to conventional preservation lungs. Mulloy and coworkers (42) added a selective adenosine 2A agonist to the perfusate in a model of one hour of warm ischemia in pigs. After procurement, lungs submitted to extra four hours of cold ischemia and then four hours of EVLP performed significantly better than lungs submitted to four hours of cold ischemia only, with less histological lung injury and lower levels of inflammatory cytokines in the bronchoalveolar lavage after single left LTx.

Lastly, some groups have moved further with the clinical use of uncontrolled DCDs. Pioneer work from the Hospital Universitario Puerta de Hierro has initially shown high incidence of PGD3 (38%), with 17% hospital mortality and 57% 1-year survival from 29 uncontrolled DCD LTxs (43). The addition of EVLP to this algorithm helped to better select this lungs and rendered no case of PGD3 in the initial 4 EVLP LTxs, with additional exclusion of four lungs with poor EVLP performance (44). More recently, Tom Egan has shown the feasibility of a similar approach in a US clinical trial, having procured and perfused two uncontrolled DCD lungs. Although one of them deteriorated on the circuit, the other one presented adequate function and was not transplanted only because there was no recipient to match blood type and size (45).

The future

The current EVLP assessment is mainly based on physiological parameters, added to lung X-ray, bronchoscopy and macroscopic evaluation. Although EVLP has provided similar results of LTx with extended criteria donor lungs compared to those with conventional ones, we still observe a small percentage of PGD3. Certainly one cannot control for recipient factors, nevertheless, the addition of biomarkers to EVLP assessment has the potential to further refine donor lung selection. Since plausible biomarker candidates have been suggested, the next barrier to clinical translation resides in the design of rapid diagnostic assays in order not only to validate but also to provide this information in a timely fashion.

Acknowledgements

None.

Footnote

Conflicts of Interest: M. Cypel was a principal investigator for the Toronto Ex-Vivo Lung Perfusion Trial sponsored by Vitrolife, a company that makes sterile solutions for organ preservation. M. Cypel is a founding member of Perfusix Inc., a company that provides ex-vivo organ perfusion services.

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Cite this article as: Machuca TN, Cypel M. Ex vivo lung perfusion. J Thorac Dis 2014;6(8):1054-1062. doi: 10.3978/j.issn.2072-1439.2014.07.12

The surgical technique of bilateral sequential lung transplantation

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Abstract: Since the first successful lung transplant performed three decades ago, the technique of lung transplantation has evolved with acceptable short- and long-term outcomes such that it has become the standard for those with end stage pulmonary disease. Herein, we describe our current favored approach and discuss some of the current areas in need of further investigation as they relate to the technical aspects of the operation.

Keywords: Lung transplantation; surgical technique; end-stage lung disease; bilateral sequential lung transplantation; single lung transplantation

Submitted Apr 01, 2014. Accepted for publication Jun 19, 2014.
doi: 10.3978/j.issn.2072-1439.2014.07.02
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.07.02

Introduction

Lung transplantation is the most effective treatment modality for end-stage pulmonary disease (1-4). The number of procedures performed continues to increase every year with an estimated 3,000 transplants being performed annually (1). The majority of transplants performed today are bilateral sequential procedures (3). This is supported by evidence within the literature that has identified a long-term survival benefit from bilateral as opposed to single lung transplantation (5,6). Operative techniques and critical care, however, continue to evolve and 1- and 5-year outcomes continue to improve (5,7).

Operative approach

The decision as to whether to use extracorporeal support during bilateral lung transplantation varies with institutional experience and with patient selection. The bulk of the decision-making should be made preoperatively and can be modified based on intraoperative hemodynamic stability. Recipients are prepared for the operating room (OR) well in advance by completing their routine studies. I personally ensure that all recipient studies are confirmed using a standard checklist for our program that includes the detailed review of all pre-operative studies. We also have a pre-operative safety checklist in addition to our institutional OR standards that ensures blood type and serology acknowledgement prior to entering the OR. This is specific to our organ transplant program and is a "hard stop" in the OR flow if the documentation is not completed correctly.

Following appropriate donor selection and communication with the procurement team at the donor site, it is paramount to engage in constructive dialogue with the anesthesiology, perfusion, and OR teams so that intraoperative needs are anticipated ahead of time. This involves a discussion regarding selection of antimicrobial prophylaxis, preoperative inhaled pulmonary vasodilators (such as nitric oxide), the likelihood of requiring cardiopulmonary support, immunosuppression induction, intravascular access, and the availability of blood products. Additionally, any patient or donor-specific nuances are reviewed.

Prior to intubation, two intravenous lines and a radial arterial line are placed. The patient is intubated with a double lumen endotracheal tube that is positioned using fiberoptic bronchoscopy. The time of induction can be very destabilizing and I make it a point to be in the room ready to intervene in case of cardiopulmonary instability. A left femoral arterial line is placed. Venous access is

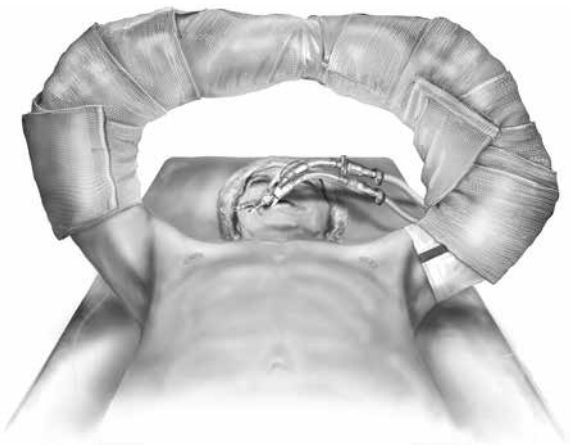

Figure 1 Patient positioning for bilateral sequential lung transplantation.

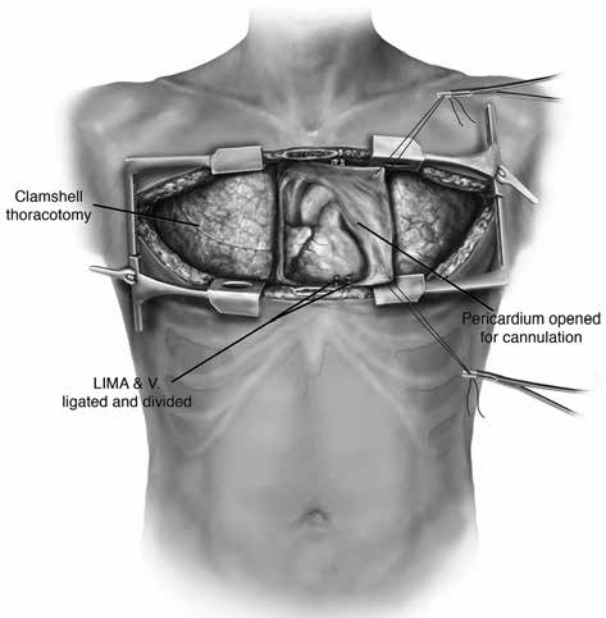

Figure 2 The approach for bilateral thoracosternotomy.

established in the right neck and left groin. If the patient is high risk or the donor lungs of marginal quality, it is prudent for the team to place the right venous neck line in the left neck in the event that post-operative extracorporeal membrane oxygenation (ECMO) may be required (the right neck would be used for a cannula during veno-venous ECMO). Placement of a pulmonary artery (PA) catheter is performed. A transesophageal echo (TEE) probe is placed in the esophagus and routine evaluation performed.

The patient is positioned supine with arms abducted, supported and padded above the head to expose both the chest and the axillary regions (*Figure 1*). The entire neck, chest, abdomen, and bilateral groins are prepped in the sterile field to allow for access to the femoral vessels in the event of the need for rapid extracorporeal support. The traditional incision used for bilateral lung transplantation is the clamshell incision, but the procedure may also be performed using separate bilateral sternal sparing anterior thoracotomies. I prefer the bilateral thoracosternotomy because of the ability to intervene with central cannulation rapidly if there is any hemodynamic compromise during the operation (*Figure 2*). This sternal-sparing anterior thoracotomy incision is a nice approach for single lung transplantation as you can easily place the patient on ECMO/CPB via the groin. Early in my practice I performed single lung transplants through posterolateral thoracotomies, but subsequently have switched to the anterior approach because of the ease of cannulation access when the patient is positioned supine.

Each of the commonly used incisions is performed by convention in the fourth (idiopathic pulmonary fibrosis) or fifth (emphysema, cystic fibrosis) intercostal space. When a clamshell incision or bilateral thoracosternotomy is performed, special care must be taken to ligate the internal mammary arteries as they can be an inconvenient source of bleeding postoperatively. Once the chest is entered, the internal thoracotomy is completed posteriorly sparing the latissimus dorsi and serratus anterior muscles. Chest retractors are placed. The mediastinal pleura is divided superiorly to the level of the mammary vein and inferiorly to the level of the pericardium.

The choice of which side should be transplanted first may be determined preoperatively by split function testing in which the worse side is transplanted first. There may, however, be other donor and recipient characteristics that dictate this decision. The lungs and chest cavity are inspected for pathologic findings. A figure-of-eight traction suture (0-silk) is placed on the dome of the diaphragm and brought out infero-medially on the external to the body. This is secured with a small clamp. The pericardium may be opened at this point or later in the case in preparation for central cannulation, to aid with hilar dissection, or to allow for intentional cardiac shifting for optimizing hemodynamics (especially for left sided anastomoses). Adhesions encountered within the chest are liberated with electrocautery. The inferior pulmonary ligament is released. The hilar dissection is then carried out and the phrenic nerve is left uninjured. Pneumonectomy is performed in a

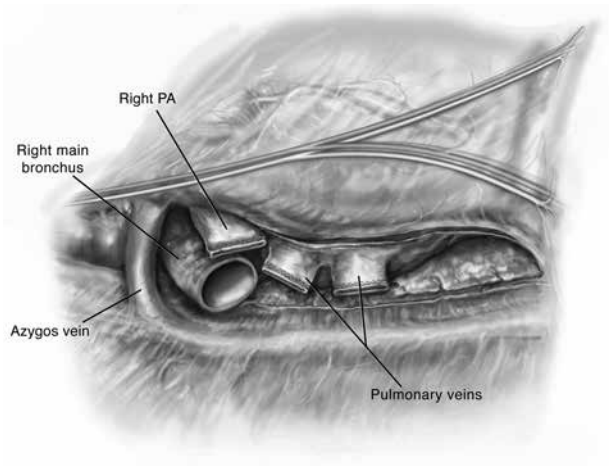

Figure 3 The view of the right hilum following recipient pneumonectomy (right side is shown).

standard fashion beginning with the division of the inferior pulmonary ligament, the sequential encircling of the PA and pulmonary veins (PV) followed by multiple firings of an endo GIA stapler staying as peripheral as possible. Before stapling the PA, it is snared down using a tourniquet for 5 to 10 minutes to assess hemodynamic stability. In the event of escalating PA pressure, the decision to use cardiopulmonary support should be made. Regardless of the circumstance, I give a small dose of Heparin (100 U/kg) systemically and keep the activated clotting times (ACTs) 160-200 once the PA is clamped. If ECMO is used then I run the ACTs 180-250. If CPB is used, the ACTs are that for standard CPB. Generally, for CPB, my preference is for central cannulation that includes an aortic cannula and a two-stage venous cannula. Of course, cannulae size and other variables are adjusted to the patient characteristics and potential need for additional cardiothoracic procedures (patent foramen ovale closure, coronary artery bypass, etc.).

For the pneumonectomy, the pulmonary vessels are divided first followed by the bronchus. On the right side the bronchus is divided immediately proximal to the takeoff of the right upper lobe. On the left side, I divide the bronchus immediately proximal to the secondary carina. During the division of the bronchus, the fraction of inspired oxygen (FiO_2) should be decreased to less than 30% and suction applied to the ipsilateral side through the double lumen ET tube so as to minimize the entrainment of high flow oxygen that could result in sparking a fire due to the simultaneous use of electrocautery. We also flood the field with CO_2. Once the pneumonectomy has been performed, the recipient lung is cultured and then sent for permanent fixation, sectioning, and pathological examination.

The hilum is then prepared by circumferentially opening the pericardium (*Figure 3*). This affords mobilization of the PVs and PA to admit clamps. The bronchus is prepared centrally and cut with an angled scalpel at the desired length. On the right side I prefer to cut at 2 rings from the carina. During this preparation the mediastinal lymph nodes are liberated such that a safe anastomosis may be performed. Bronchial arteries are ligated with cautery and clips to prevent significant bleeding. Denudation of the recipient bronchus should be avoided to prevent ischemic complications (8-10). Any secretions within the bronchus are suctioned liberally and the double lumen endotracheal tube is adjusted appropriately. The pleural space and bronchus are irrigated liberally with antibiotic-containing solution. The amount and content of irrigation is typically recipient and center-dependent.

Back table preparation is performed to ready the donor lungs for implantation. With the graft on ice, the bronchus, PVs, and PA are prepared. The donor bronchus is cultured. Extra tissue from the procurement is removed sharply or with electrocautery. The donor bronchus is trimmed to within approximately 1-2 rings from the lobar takeoffs. We use crushed ice to keep the recipient thoracic cavity cool during the implantation with a "phrenic pad" placed *in situ* to protect graft from warming and from direct contact with the body wall. The implantation is then conducted sequentially beginning with the most posterior anatomical structure, the bronchial anastomosis (*Figure 4*). The bronchial anastomosis is completed using a running 3-0 polypropylene suture which begins with the membranous portion of the airway and ends anteriorly on the cartilaginous portion. The anastomosis is performed in an end-to-end fashion taking great care to achieve membranous-to-membranous and cartilaginous-to-cartilaginous apposition. My preference is to reinforce the suture line at 10 and 2 o'clock with two additional 3-0 polypropylene stitches thereby locking the continuous suture line in place. The anastomosis is immediately inspected using bronchoscopy. In our experience, we routinely tack an edge of intervening donor pericardium to separate the bronchus from the PA.

The PA anastomosis is fashioned next following the infusion of 500-700 mL of pulmoplegia into the PA using a handheld antegrade cannula. This flows from retrograde exiting through the PV and is recirculated using "cell saver". A Satinsky clamp is placed proximally on the PA

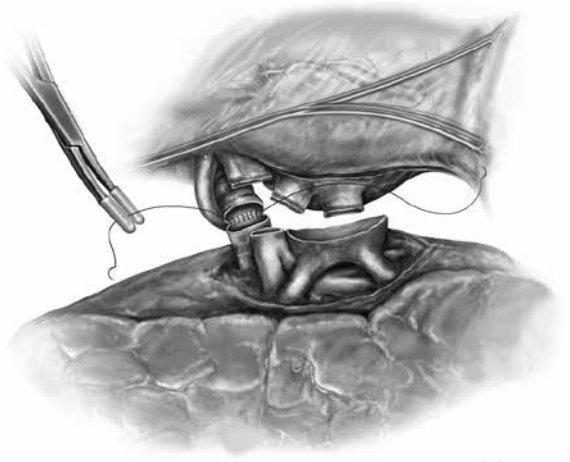

Figure 4 The bronchial anastomosis.

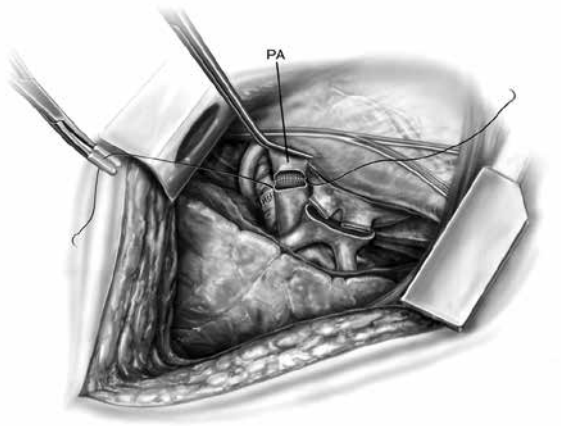

Figure 5 The pulmonary artery (PA) anastomosis.

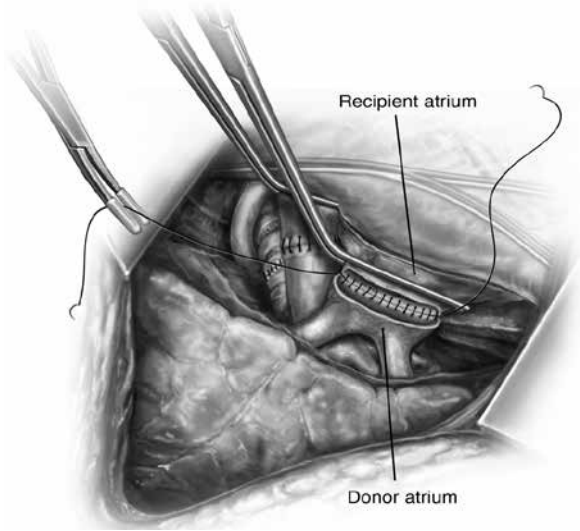

Figure 6 The left atrial anastomosis.

and the staple line is removed. The donor PA is trimmed to an appropriate length. Care must be taken to not leave the donor PA too long or too short such that problems with kinking or tearing are avoided respectively. The PAs are aligned and anastomosed using a continuous 5-0 polypropylene suture (*Figure 5*). At the completion of the suture line, they are clamped and not secured until later.

The left atrial anastomosis is next and this is aided by circumferential mobilization of the left atrium within the pericardium. A large Satinsky clamp is placed on the body of the left atrium. The staple lines of the superior PV and the inferior PV are excised and connected creating a recipient cuff for anastomosis. An endothelial to endothelial, end-to-end anastomosis is then performed using a running 4-0 polypropylene suture (*Figure 6*). Attention is taken to include the intima and exclude the muscle from the suture line. As the anastomosis nears completion, the anesthesiologist should administer 250-500 mg IV methylprednisolone.

We do not immediately knot down the anastomoses and instead allow for flushing and de-airing using 500-700 mL of "hotshot pulmoplegia" administered using a handheld cardioplegia cannula in antegrade fashion, thereby reperfusing the allograft. The Satinsky clamp on the PV is then partially opened to deair and the PV knot is tied. The PA is then unclamped over the course of 5-15 minutes and the suture line is secured. This affords controlled low pressure perfusion of the lung. Ventilation with minimal FiO_2 (preferably less than 30%) is initiated by hand and then by mechanical ventilation. A gentle Valsalva may be performed to overcome atelectatic de-recruitment and allow for efficient expansion of the lung. At this point the chest is irrigated and the bronchus tested for leak under saline immersion to a pressure of 25-35 cm H_2O. Once satisfied with this, positive end-expiratory pressure (PEEP) is set at 8-10 cm H_2O and the patient is ventilated under pressure control or with tidal volumes approximately 5-7 mL/kg donor weight. Intraoperative TEE is utilized to evaluate for de-airing and gradient measurement across the PVs and PA. The suture lines are inspected for hemostasis and once satisfied with this, the patient is allowed to recover during this time for 10-15 minutes before the opposite side is addressed in an exact analogous fashion.

By convention, we place three chest tubes in each pleural cavity. A large bore chest tube is positioned anteriorly in the chest. A 24F Blake drain is placed along the diaphragm and posteriorly towards the apex in the chest. A third large bore right angled chest tube is placed posterolaterally.

This is the same for each chest. If the pericardium was opened as I do in the vast majority of transplants, a 24F Blake drain is placed in the pericardium. The bilateral thoracotomy incision is closed using interrupted #5 Poly (ethylene, terephthalate) suture in a figure-of-eight fashion. The sternum approximated using three number 6 sternal wires. The pectoral fascial layer, the subcutaneous layer, the subdermal layer, and the skin are reapproximated with absorbable suture. Recently, we have been much more liberal with staples for skin closure. If the lungs are oversized, there is significant PGD, or hemodynamic instability, we leave the chest open according to that method previously described (11).

The double lumen endotracheal tube is exchanged for a single lumen endotracheal tube and bronchoscopy is performed for pulmonary toilet immediately post procedure. During this time a nasoenteric feeding tube is also placed with the added benefit of performing this under endoscopic control of the airway to avoid the inadvertent placement of the feeding tube within the airway. We typically use conservative FiO_2 concentration of 40% in the immediate postoperative phase to avoid theoretical risk of free radical-induced oxygen toxicity and PEEP of 10. Adjuncts such as Nitric oxide and epoprostenol should be weaned off expeditiously in the first 12-24 hours postoperatively to allow for prompt extubation.

Areas of debate related to technique

There have been a number of unsuccessful efforts in the past at reaching a consensus regarding various technical aspects of lung transplantation. Attempts, for example, made to reduce the incidence of the risk of airway complications have resulted in the varying popularity of a number of techniques (12-14). This has included telescoping of the bronchial anastomosis, the use of vascularized pedicle flaps, and even bronchial artery revascularization (15-21). We believe that the increased technical detail and variation in experience with these various steps has not allowed for any consensus beyond what we have described in this report. We also recognize that the intraoperative use of pulmoplegia before and after the fashioning of the pulmonary anastomoses is not a universally accepted practice. I have performed transplants both ways and observed no distinct differences. Thus, the use of pulmoplegia is an area deserving of further investigation.

The debate between the use of interrupted versus continuous suture techniques for the bronchial anastomosis continues to garner supporters on either side of the argument. FitzSullivan and colleagues described the use of continuous suture on the membranous bronchus and interrupted figure-of-eight suturing of the cartilaginous bronchus (14). Weder and colleagues on the other hand, prescribe the use of interrupted suture circumferentially around the entire anastomoses (22). Both groups, as is typically the case, reported satisfactory results and a reduction in airway complications. I perform a modified version of the continuous anastomosis placing two additional interrupted sutures for two reasons. One, it allows me to rest more easily knowing that there are additional sutures and the continuous suture line does not depend on one single running polypropylene suture. Two, if the anastomosis falls apart, I can blame myself such that the trainee that typically sews the continuous suture line is alleviated of the responsibility for this complication.

There has also been a growing trend in the use of lobar lung transplantation which has been fueled by the paucity of donors and the increasing need to match larger donors with smaller recipients. This has resulted in an increased consideration for lobar lung transplantation and outcomes have been acceptable where the simpler procedure of graft reduction was not considered a durable option (23-25).

Conclusions

The technique of bilateral sequential lung transplantation has evolved over the years to make it relatively safe operation when combined with careful pre-operative candidate selection, careful donor selection, and advances in critical care. The improvement in early patient survival has been achieved by a reduction in the overall rate of PGD to 5-15%. Post-operatively, severe PGD as marked by hypoxia, pulmonary edema, elevated PA pressures, and poor compliance needs to be recognized early and intervened on. We advocate for early institution of veno-venous (V-V) ECMO when recipients are deteriorating and require FiO_2 >70% to better manage the patient and avoid further injury from barotrauma.

The hallmark of post-operative care is a team approach which should mirror that of the team approach to patient selection. This includes involving anesthesia with relationship to pain control as the placement of paravertebral catheters may be of substantial benefit in recovery. There is no doubt that a dedicated intensivist with experience in cardiothoracic surgery is critical to managing fluids, hemodynamics, and optimizing the outcome of

end organ perfusion. We have also instituted a clinical pathway to aim towards early extubation and recovery that involves multidisciplinary input from pulmonary medicine, pharmacists, transplant infectious disease, cardiopulmonary rehab, etc. This has led to tremendous progress in lung transplantation over the past several years with 1-year and 5-year survival rates comparable to those of other solid organs. Although organ supply is remains limited, the current era of *ex vivo* lung perfusion (EVLP) holds great promise for increasing the number of organs available, (re)assessment of graft performance, and potentially repair/reconditioning of donor organs (26,27). This is an exciting time for lung transplantation and investigations into some of the newer areas of lung transplantation, such as EVLP, should afford improved understanding of the nuances of the surgical technique and ultimately translate into improved early and late outcomes for patients with end-stage lung disease.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Hayanga JW, D'Cunha J. The surgical technique of bilateral sequential lung transplantation. J Thorac Dis 2014;6(8):1063-1069. doi: 10.3978/j.issn.2072-1439.2014.07.02

Bridge to lung transplantation and rescue post-transplant: the expanding role of extracorporeal membrane oxygenation

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Abstract: Over the last several decades, the growth of lung transplantation has been hindered by a much higher demand for donor lungs than can be supplied, leading to considerable waiting time and mortality among patients waiting for transplant. This has led to the search for an alternative bridging strategy in patients with end-stage lung disease. The use of extracorporeal membrane oxygenation (ECMO) as a bridge to lung transplantation as well as a rescue strategy post-transplant for primary graft dysfunction (PGD) has been studied previously, however due to initially poor outcomes, its use was not heavily instituted. In recent years, with significant improvement in technologies, several single and multi-center studies have shown promising outcomes related to the use of ECMO as a bridging strategy as well as a therapy for patients suffering from PGD post-transplant. These results have challenged our current notion on ECMO use and hence forced us to reexamine the utility, efficacy and safety of ECMO in conjunction with lung transplantation. Through this review, we will address the various aspects related to ECMO use as a bridge to lung transplantation as well as a rescue post-transplant in the treatment of PGD. We will emphasize newer technologies related to ECMO use, examine recent observational studies and randomized trials of ECMO use before and after lung transplantation, and reflect upon our own institutional experience with the use of ECMO in these difficult clinical situations.

Keywords: Lung transplantation; extracorporeal membrane oxygenation (ECMO); primary graft dysfunction (PGD)

Submitted Mar 01, 2014. Accepted for publication May 09, 2014.
doi: 10.3978/j.issn.2072-1439.2014.06.04
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.06.04

Introduction

The prevalence of lung transplantation has increased significantly over the last few decades, especially in the treatment of end stage lung diseases such as chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), and cystic fibrosis (CF) (1). In 2012, over 3,640 lung transplants were recorded in the registry of the International Society for Heart and Lung Transplantation, up from 3,395 the year before (1). Early survival following lung transplantation has improved over the years with 1-year survival approaching 79% (1). Unfortunately, the long-term success of lung transplantation has only seen a modicum of improvement, with median survival for the most recent era averaging 6.1 years (1).

Due to its modest successes and changing demographics, waiting time for lung transplantation continues to be an issue as the need for donor organs far exceeds their availability (2). While the implementation in the United States of the lung allocation score (LAS) in 2005 has helped to prioritize patients in the most urgent need for transplantation, roughly 500 patients continue to die while awaiting a lung transplant every year (3-5). The resulting estimates of mortality for patients on the waitlist is concerning, and has raised considerable interest in looking for alternative bridging strategies for patients with end-stage lung disease awaiting transplantation (2).

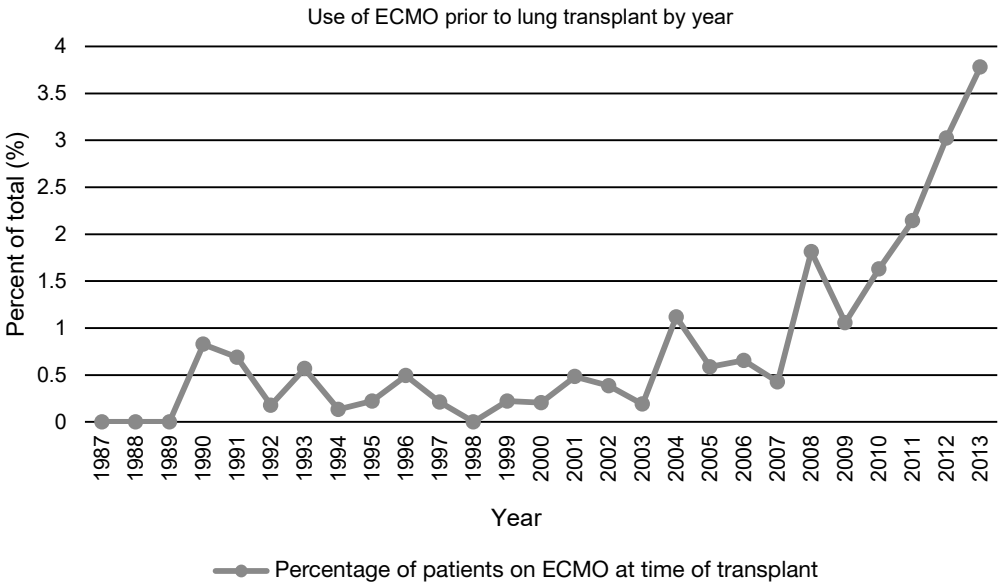

Figure 1 Percentage of patients on ECMO at time of transplant by year. Data obtained from the United Network for Organ Sharing (UNOS) database 1987-2013. Only patients with no previous transplant were included. ECMO, extracorporeal membrane oxygenation.

Utility of ECMO

Extracorporeal membrane oxygenation (ECMO) is a complex technique that allows for respiratory and/or cardiac support in critically ill patients (6). There are many indications for the implementation of ECMO, including adult respiratory distress syndrome (ARDS), inability to wean from cardiopulmonary bypass, and cardiogenic shock, among others (7). It can be used both in a veno-venous (VV) circuit for pure pulmonary support as well as a veno-arterial (VA) circuit for concomitant cardiac support (8-10). Cannulation strategies and implantation techniques vary tremendously based upon the local environment, resources and patient needs (6-8). Because of the technical expertise required and considerable financial costs, its use has been limited to patients with a high risk of mortality and whose underlying disease process is either reversible or as a short-term "bridge" to more definitive therapy (11).

Over the last several years, the use of ECMO as a bridge to lung transplantation has gained significant attention in the management of patients with severe end-stage lung disease (9,12). Historically, ECMO use in this setting has been associated with poor outcomes which led many to condemn the practice (13,14). However, in recent years, technical advances have resulted in the extended use of various extracorporeal life support (ECLS) devices, such as ECMO, in the management of patients presenting with acute respiratory failure with significant improvement in outcomes (15). Furthermore, the implementation of the LAS has led to decreasing waiting times for lung transplantation (16). Combined, this has also led to a reinvigoration in the use of ECMO as a bridge to lung transplantation. In a study of more than 9,000 patients from the UNOS database from 2005 to 2011, roughly 1% of pulmonary patients were bridged to transplant with ECMO support (5). These numbers have continued to grow since then as an increasing number of single-center studies have demonstrated the utility and successful outcomes associated with ECMO as a bridging strategy to lung transplantation (*Figure 1*) (17-23).

Historical challenges

There has been significant variability in the use of ECMO as a means of bridging patients to lung transplantation over its short history (*Figure 2*). Hill *et al.* first reported the use of ECMO as a treatment modality for the management of cardiopulmonary failure in 1972 (24). Shortly thereafter in 1975, ECMO was described as a means of bridging a patient to lung transplantation, however further use was impeded by poor initial outcomes (13,14,25). Unacceptable post-transplant survival following pre-operative ECMO was likely

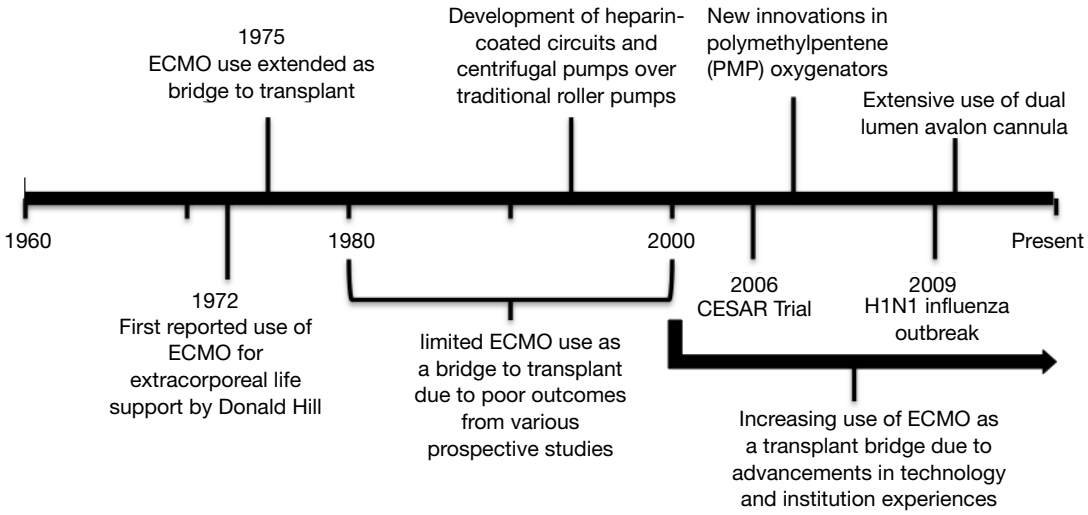

Figure 2 Historical points of interest in the use of ECMO as a bridge to lung transplantation. ECMO, extracorporeal membrane oxygenation.

related to both the severity of the patient's illness and the technological inadequacies of early ECMO systems (17). Furthermore, it was traditionally regarded that ECMO use pre-transplant was associated with impaired bronchial anastomotic healing that contributed to the morbidity and mortality in lung transplant recipients (25). In addition, the results of a randomized, prospective study in 1979 demonstrating no survival benefit from ECMO in a non-lung transplant cohort of patients with acute respiratory failure further contributed to this declining use (18).

For the next two decades, the use of ECMO as a bridge to lung transplant was only sporadically used and limited to a few centers with mixed outcomes. However, significant improvements in ECMO-related technologies were made during this time period and data accrued slowly that challenged earlier preconceptions about the utility of ECMO (19,20). For example, during the 2009 H1N1 influenza outbreak, ECMO gained special attention by successfully managing a significant proportion of patients with severe acute respiratory failure (19). Furthermore, the *Conventional Ventilation or ECMO for Severe Adult Respiratory Failure (CESAR)* trial was conducted in the United Kingdom, and demonstrated a significant survival benefit of ECMO compared to conventional management for patients with severe ARDS (20).

ECMO as a rescue strategy post-transplant

Background

In lung transplantation a renewal of interest in ECMO was first seen for severe primary graft dysfunction (PGD) following lung transplantation and this remains the most common indication for its use after transplant (21,22). PGD is a syndrome consisting of lung injury during the first 72 hours following lung transplant defined as a decreased PaO_2/FiO_2 ratio and the presence of diffuse infiltrates on chest X-ray (23,26). As institutional experience with ECMO accrued, several isolated studies and case reports explored the use of ECMO as a rescue strategy in the treatment of PGD post lung transplantation, and as a bridge to redo lung transplant in select patients with intermittent successes (27,28). About 5% of lung transplant procedures require ECMO support for PGD or early complications (21). Many interventions have been studied to try and ameliorate the effects of PGD after transplant, including experimentation with inhaled nitric oxide and prostaglandins (29,30). However, none of these have been successful in significantly altering the rates of clinically important Grade 3 PGD, which hovers at about 17% according to results of the Lung Transplant Outcomes Group (26). According to this multi-institutional study, grade 3 PGD was associated with a 23% absolute increase in the risk of death within one year of transplant, indicating it's continued overall impact on transplant survival (26).

Indications

An important question remains regarding when to employ ECMO after transplantation. Enhanced safety combined with increased experience has led to earlier

deployment of ECMO circuits to support patients after lung transplantation (31). The goal should be to avoid or minimize the detrimental effects of ventilator support for PGD secondary to elevated airway pressures or high inspired oxygen concentrations. Firm guidelines vary from center to center, but we recommend initiating ECMO support when ventilatory requirements reach a peak inspiratory pressure of 35 cm H_2O or F_iO_2 surpasses 60% in order to minimize lung injury from aggressive mechanical ventilation and oxidative stress. When necessary, the delayed initiation of ECMO after transplantation greater than 48 hours has been associated with worse outcomes, and this is consistent with our own experience that favors prompt initiation of ECMO (32).

Outcomes

Our group and others have reported on utilizing ECMO to support the recipients who suffer from severe PGD. Survival in this group of patients was surprisingly good when supported with VV ECMO, especially considering the lethality of severe PGD (22). The mean reported ECMO duration post-transplant is varied, but most studies have reported between 2 to 8 days (21,28,33). One study demonstrated successful use of ECMO for 3 weeks prior to a redo lung transplantation, however others have demonstrated that prolonged ECMO duration post-transplant is associated with high mortality (28,34). Nonetheless, it provides a means of treatment in patients who suffer from PGD post-operatively who would otherwise succumb quickly. We have reported a 96% success rate in weaning recipients from VV ECMO following transplant, with a 30-day survival of 82% and 1-year survival of 64% (22). Some centers report success with both VA and VV ECMO for these patients. However, our experience has been that VV ECMO should be preferred due to a decrease in complications and greater survival when compared to patients supported with VA ECMO (21).

ECMO as a bridge to transplant

Background

In the last several years, there has also been a continued push at individual centers to reexamine lung transplantation in patients on ECMO. Numerous reasons are cited for this including a benefit in weaning patients off of mechanical ventilation (which is also associated with increased post-operative mortality) as well as allowing patients with acute respiratory failure to be transported to centers with transplant services from those without (35-37). Others use recent advancements in technology as an argument for reexamining this issue. For instance Jackson *et al.* list three major recent advancements in ECMO: the development of the polymethylpentene (PMP) oxygenator, the use of heparin coated circuits, and the use of centrifugal pumps over traditional roller pumps (25). We would add portability and the dual-lumen cannula to this list and emphasize that together these advances have led to the ability to minimize anticoagulation needs and likely result in much less hemolysis and activation of blood components traveling through the circuit.

Indications

Although multiple centers have published with regards to their successes transplanting patients following the use of VV ECMO, there are no universally accepted indications for this practice (10,27,38). Careful patient selection for lung transplantation after ECMO is imperative to maximize outcomes and ensure appropriate resource allocation of scarce donor lungs. Current recommendations are based on institutional experience. Much of the earliest use of ECMO as a bridge to lung transplantation was for patients with PGD requiring retransplantation, and therefore this was seen as an early indication (39). Since that time however, improving outcomes have led to the use of ECMO bridging in patients without prior transplantation (13,39). Most studies recommend the use of this practice primarily in younger patients who suffer an acute decompensation in a chronic pulmonary process, not for acute respiratory distress syndrome (9,39). Furthermore, these patients should have had reasonable functional status prior to their acute episode (35,39). However, there has been anecdotal success in bridging previously healthy young patients to transplant when they suffer irreversible lung injury acutely.

Contraindications

Current contraindications for lung transplantation following ECMO are also based on institutional experience (*Table 1*). For instance, Lafarge *et al.* recommended that renal failure be considered a contraindication for transplantation following ECMO due to the intraoperative death of a patient who had pre-transplant anuric renal failure (10). Toyoda *et al.* recommended this be expanded to

Table 1 Contraindications (both absolute and relative) to bridging to lung transplant with ECMO (9,10,37,39,40)

Absolute contraindication
Untreated infection
Organ failure (other than pulmonary)
Recent malignancy
Active substance abuse
Poor social support system
History of nonadherence
Relative contraindication
Advancing age
Small institutional experience
Poor pre-ECMO functional status
Severe obesity (BMI >30)

ECMO, extracorporeal membrane oxygenation.

Table 2 Overview of recent single and multi-institution studies reviewing outcomes following lung transplantation after ECMO

Study	Number of patients	1-year survival (%)
Toyoda *et al.* [2013] (9)	24	74
Hoopes *et al.* [2013] (12)	31	93
Anile *et al.* [2013] (44)	7	85.7
Nosotti *et al.* [2013] (43)	11	85.7
Lafarge *et al.* [2013] (10)	30	66.5
Bittner *et al.* [2012] (27)	27	33
Gottlieb *et al.* [2012] (42)	60	57
Lang *et al.* [2012] (38)	34	60
Hämmäinen *et al.* [2011] (13)	13	92

ECMO, extracorporeal membrane oxygenation.

any organ failure including liver failure (9). Other studies including those by Bermudez *et al.* and Mason *et al.* discuss how pre-transplant ECMO populations tend to be younger, likely demonstrating inherent selection biases (9,37,39). Further research is necessary to determine if increased age is an absolute or relative contraindication. Multiple studies have also documented that their institutional outcomes have improved over time, likely secondary to a mixture of newer technology/protocols as well as extensive experience (39). As lower-volume centers are often limited in experience, ECMO use as a bridge to transplant should likely be limited at these centers until standardized best-practice protocols have been developed to optimize outcomes (9). Lastly, traditional contraindications to lung transplantation including uncontrolled or untreated infection, recent malignancy, significant coronary artery disease, and active substance abuse among others continue to be contraindications to the use of ECMO-bridged transplantation (40).

Outcomes

Although several trials have evaluated the outcomes of ECMO in severe respiratory failure, very few have examined in isolation, the utility and role of ECMO as a bridge to lung transplantation. Current literature is limited to several single center retrospective studies advocating for the use of ECMO as an alternative "salvage" therapy in patients with end-stage lung disease (9,10,12,13,27,35,36,38,41-44). Most of these analyses were composed of a mixture of ambulatory/extubated patients and sedated/intubated patients. A summary of these studies can be found in *Table 2*. One year survivals ranged from 33-93%, many of which are better than that reported previously (9,10,12,13,17,27,37,38,43,44). Moreover, diagnoses in these groups varied, but overall CF and idiopathic pulmonary fibrosis (IPF) had a higher prevalence while COPD had a lower prevalence than that of the general lung transplant population (1,9,10,12,35,36,38,41,42). As an indication of the changing times, there has been over a 200% increase in lung transplantation in patients on ECMO between 2009-2013 (*Figure 1*).

The discrepancy noted in survival outcomes among the above referenced studies is unclear; however, we speculate that this may be attributable to the nature of ECMO used, institutional differences in cannulation strategies, disparate wait list times among centers, and the extent and severity of post-transplant complications such as PDG. Furthermore, several studies have also shown that although high acuity lung transplant patients who are bridged with ECMO have increased risk for short-term mortality compared to the average lung transplant recipient, these high-risk recipients have better overall outcomes when performed at high volume centers (5). It is likely that this success is secondary to the extensive experience and technological capabilities in managing the complexities associated with ECMO at high volume centers. It may also be secondary to shorter waiting times at these high volume centers subsequently leading to a shorter pre-transplant ECMO duration and improved survival.

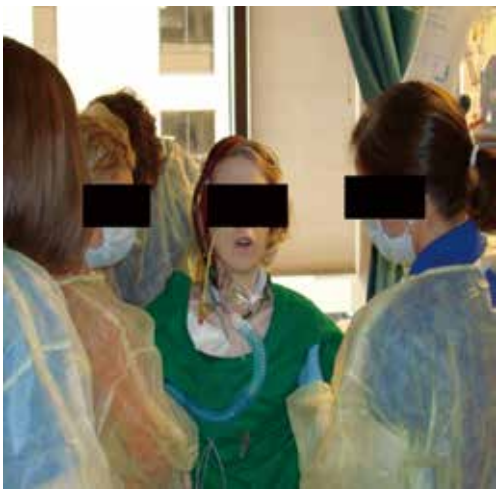

Figure 3 Demonstration of a patient ambulating on VV ECMO with a dual lumen cannula in the right internal jugular vein. VV, veno-venous; ECMO, extracorporeal membrane oxygenation.

Newer treatment modalities

With the advancement of technology and increase in institutional experience in the past few years, newer and more promising strategies of incorporating the use of ECMO as a bridge to transplant have been developed. For instance, Fuehner *et al.* examined outcomes using ECMO as a bridge to transplantation in patients who were awake and spontaneously breathing. Compared to the conventional mechanical ventilation strategy, patients who received "awake" ECMO as a bridge to transplant and made it to transplantation had significantly better survival at 6 months (80% versus 50%), and had shorter postoperative hospital stays (although not to statistical significance) (41). The authors hypothesize that the main benefit of this "awake" ECMO is the avoidance of prolonged sedation and intubation and its associated complications (41). The authors further postulate that future successes in this arena could lead to a "destination therapy" much like that seen with left ventricular assist devices (41).

Other recent advances including low-resistance gas exchange membranes, high-durability centrifugal blood pumps, heparin-coated tubing, and improved cannulation strategies have resulted in a much safer medical device compared to those in use a few years ago (45,46). Newer devices are also increasingly smaller and lightweight. The new "Cardiohelp" by Maquet Cardiopulmonary is light enough to be carried by the patient, and also can simultaneously measure patient vitals, venous oxygen concentration, and hemoglobin (47,48). Haneya *et al.* reported on its use in 22 patients with a survival rate of 68.2% (47). Further advantages of these smaller systems include easier inter-facility transport of patients, which once again can allow transport of a patient to a transplant center when indicated (48).

Our institutional experience

Taking the concept of awake ECMO one step further we recently published on our institutional experience with pre-operative ECMO in bridged patients able to perform active rehabilitation. This experience included nine patients, all of whom survived through 1-year post-transplant (17). The patients who were able to undergo active rehabilitation while awaiting lung transplantation on ECMO demonstrated shorter post-transplant ventilator duration and hospital lengths of stay. This is due to the absence of post-transplant myopathy secondary to participation in active rehab. Our rehab protocol begins with the weaning of sedation and ventilator settings. Most of the patients will require tracheostomy, which is performed early in the process or at the time of ECMO cannulation. A few patients may be extubated while on ECMO. Resistance and stretching exercises follow once awake. The patients then progress through sitting, standing, and eventually ambulation. At least two formal rehab sessions are performed each day with staffing consisting of a physical therapist, ECMO specialist, respiratory therapist and 1-2 bedside nurses. Although resource intensive, patients have demonstrated the ability to walk up to 400 meters during one session and outcomes appear to be considerably improved.

Technical aspects of ECMO

Historically, extracorporeal support required dual cannulation, such as the femoral and internal jugular veins for VV or femoral vein and artery for VA ECMO. Femoral cannulation sites may increase the risk of infection and impede patient mobility. Therefore, whether it is for bridging to transplant or support after transplant, our most commonly employed ECMO strategy now involves a VV technique utilizing a dual-lumen cannula (Avalon Maquet) in the right internal jugular vein (*Figure 3*) (49). However, many other cannulation strategies are possible for both VV as well as VA ECMO and are oftentimes dictated by patient

anatomic limitations or other factors (6,9,35). For active rehabilitation on VA ECMO, our most common approach is to sew a 6 to 8 mm vascular graft to the right axillary artery with a 21 to 23 mm venous cannula in the right internal jugular vein for drainage. Based on our experience, if at all possible we recommend a cannulation and ICU management strategy that will allow for active rehabilitation while awaiting lung transplantation on ECMO support.

Complications related to ECMO

Complications resulting from ECMO use are common, and depend on the type of ECMO technique (VA or VV) as well as the cannulation strategy used (7,9). Usual complications include bleeding, infection, and renal failure as well as less common complications including gas embolism, stroke, and limb ischemia (8,11,50,51). Bleeding is perhaps the most commonly reported complication ranging from 5-79% in the literature (11,52). Its cause is multifactorial, both secondary to iatrogenic anticoagulation necessary for ECMO as well as thrombocytopenia and fibrinolysis occurring because of contact with the ECMO circuit (11,53). Treatment is best performed through prevention, and modern circuits as described above allow users to reduce the requirement for systemic anticoagulation (11,53).

Although difficult to predict, it is pertinent to quickly identify and treat these complications to reduce associated mortality. Limb ischemia is a specific complication for which prompt diagnosis and action can improve outcomes. Occurring in 13-25% of VA ECMO patients cannulated through the femoral artery, its incidence can be reduced through use of a secondary distal catheter to increase distal limb perfusion, or through reliance on VV ECMO whenever possible to avoid arterial cannulation (54,55). Proper anticoagulation can also prevent emboli formation in the ECMO circuit. When limb ischemia is diagnosed early, prompt treatment can avoid permanent limb injury and reduce the amputation rate (54,56).

Conclusions

Lung transplantation is now considered an appropriate therapeutic option for the treatment of patients with end-stage lung disease (5). However, given the paucity of available donors, there is still significant mortality for patients on the waiting list (2). Historically, the use of extracorporeal circulatory support such as ECMO was considered to be a contraindication to lung transplantation due to poor outcomes (14). However, in recent years, this trend is evolving as more institutions look to optimize the safety and efficacy of their ECMO strategies as a means of bridging high-risk and high-acuity patients for lung transplant (44).

As larger institutional studies are performed, a clearer picture as to the outcomes of ECMO use is emerging. Some are already calling for a randomized multicenter controlled trial to help give an answer to this question (57). However, there are still many unanswered questions remaining and a randomized trial of adequate size is unlikely to ever be successfully performed. Therefore, it will be up to the lung transplant community to determine issues such as how the need for pre-transplant ECMO should weigh in to organ allocation, or what the appropriate indications and patient populations to bridge to lung transplantation should be. No doubt that as technologies continue to improve we will be obliged to revisit these questions, as well as many others periodically.

Modern experience with ECMO and reported institutional experiences on survival challenge historical assumptions about the treatment of end-stage lung disease and suggest that "bridging" to transplant with ECMO is both technically feasible and logistically viable. What is clear at this point in time is that continued advances in the technologies and further research will help determine how best to include ECMO as a bridging strategy for lung transplantation.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Gulack BC, Hirji SA, Hartwig MG. Bridge to lung transplantation and rescue post-transplant: the expanding role of extracorporeal membrane oxygenation. J Thorac Dis 2014;6(8):1640-1653. doi: 10.3978/j.issn.2072-1439.2014.06.04

Airway anastomosis for lung transplantation

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Abstract: Lung transplantation (LT) is the only viable option for a selected group of patients with end stage pulmonary diseases. During the recent years satisfactory results in terms of long-term survival and quality of life have been achieved with improvements in surgical technique, immunosuppression and perioperative management. Since the beginning, the airway anastomosis has been considered crucial and significant efforts have been made to understand the healing process. A number of experimental studies allowed improving the surgical technique by modifying the technique of suturing, the anastomotic protection and type and dose of immunosuppression, reducing the risk of airway complications. Furthermore, a huge progress has been made in the management of such complications. Early diagnosis of bronchial complications and their prompt and correct management are crucial to achieve long-term survival.

Keywords: Lung transplantation (LT); bronchial anastomosis; airway complications

Submitted Sep 04, 2015. Accepted for publication Jan 06, 2016.
doi: 10.3978/j.issn.2072-1439.2016.01.67
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2016.01.67

Introduction

Lung transplantation (LT) is currently considered the only viable option for a selected group of patients with end stage pulmonary disease not responding to medical or surgical therapies and with a life expectancy of less than 2 years. The most common indications for LT include four groups of diseases: obstructive, restrictive, septic and vascular; survival varies according to the underlying disorder, with better results for cystic fibrosis and emphysema and worse for idiopathic pulmonary fibrosis. During the last 20 years the technical aspects of the procedure, organ preservation, perioperative management and immunosuppression have been dramatically improved.

Since the early days of LT, healing of the airway anastomosis has been considered the Achilles' heel limiting survival (1-3). A number of experimental studies have been performed to understand the causes of healing impairment and to reduce the risk of catastrophic events related to airway complications (4,5). The development of the bilateral sequential technique with separate bronchial anastomoses has been somehow forced by the high rate of anastomotic tracheal dehiscence (25%) leading to fatal events (6,7) reported for the en-bloc procedure with tracheal anastomosis. Furthermore, LT represents an exception in the transplantation world: in fact, the lung is the only organ in which the arterial systemic blood supply (bronchial arteries) is not routinely restored during the transplant and a network of bronchial circulation around the anastomosis is detectable only after 4 weeks (8). This detail may explain the frailty of the anastomotic site and why different factors including donor management, high—dose steroids administration, immunosuppression therapy, surgical technique and perioperative management can affect its correct healing.

Historical background

The current knowledge of the process of airway healing

after LT is based on experimental studies performed during 1980s by the Toronto Lung Transplant Group on auto-transplanted lungs in dogs. Initially, they focused on the effects of steroids and azathioprine (the only immunosuppressive drugs used at that time) on the breaking strength of bronchial anastomosis; they reported that only steroids were responsible for the impaired healing while azathioprine had no effects (3). The introduction in the clinical practice of cyclosporine A (CSA) allowed to dramatically decrease the rate of airway complications in the same animal model. These data were confirmed at scanning electron microscopy, showing normal collagen formation at the anastomotic site in animals receiving CSA (4). These studies contributed to reduce the use of steroids before and after LT to avoid impairing of the healing process. However, further studies demonstrated that the administration of steroids plays an important role to prevent rejection and, at ameliorate the patency of microcirculation in case of reperfusion injury (9,10). Low dose steroids also contribute to improve healing of the anastomosis in a non-transplant setting (11). For these reasons they are still included in the immunosuppressive regimen. However, their dose should be reduced as much as possible before the transplant (12).

The effects of the interruption of the bronchial circulation have long been debated. Early studies performed in 1960s showed that if the bronchial vessels are not anastomosed a higher rate of bronchial complications is observed (13,14). However, subsequent studies showed that a fine network of bronchial circulation is detectable starting from the fourth week (8) and that an early network of vessels surrounding the anastomosis is already present after 12–14 days (15). This data confirms previous reports stressing that the first two postoperative weeks are crucial to prevent airway complications (16). Based on these reports, the Toronto Group proposed to buttress the anastomosis with an omental pedicle flap to reduce the early ischemic time and enhance the microcirculation (17,18). They demonstrated that with such technique, after 4 days a network of multiple capillaries originating from the omentum surrounds the bronchus and supports healing. Although this technique has initially met a large consensus, it has been progressively abandoned in favor of new and technically easier strategies to wrap the anastomosis as the use of the intercostal muscle (19,20) or the peribronchial tissue (21).

Evolution of technical details

Since bronchial anastomosis complications can be catastrophic and significantly affect outcome, the technical aspects of suturing have been repeatedly modified and simplified since the early days. The bronchial anastomosis was initially performed after the vascular anastomoses due to the lateral decubitus of the patient on the operatory table; the cartilaginous portion was completed first with interrupted absorbable sutures, followed by the membranous portion. At the end, the omentum was transposed in the chest and wrapped around the suture line (22). The supine position on the operatory table forced to perform the bronchial anastomosis first, starting from the membranous portion.

Due to the peculiarity of the airway vascular support (low pressure circulation from the pulmonary arteries and systemic pressure circulation from bronchial arteries; both divided at time of transplantation), the length of the donor bronchus has been historically considered crucial to prevent airway complications. The donor bronchus is usually transected no more than one or two rings above the lobar carina to minimize the area of ischemia (23). More recently, several reports suggested that an even shorter length of donor bronchus (close to the lobar carina) might further reduce bronchial ischemia (24-26). This modification significantly contributed to decrease the rate of airway complications, independently from the surgical technique used to perform the anastomosis. However, the excessive shortness of the donor bronchus could create problems to treat major complications in case they occur; in fact, in such situation, mechanical dilation or stent placement might be difficult and sleeve lobectomy or redo transplantation might become the only available options (25).

Bronchial artery revascularization with microsurgery techniques has been proposed to improve healing (27). Although this approach allows full restoration of the bronchial circulation, the technical difficulty and the additional operative time have limited worldwide spreading.

The surgical technique for bronchial anastomosis has been repeatedly modified and even now there are differences between centers. Even the type of suture material is still debated (absorbable *vs.* non-absorbable). The classic technique proposed by the Toronto group was an end-to-end anastomosis with an absorbable 4/0 running suture on the membranous part and single or figure-of-eight stiches for cartilaginous wall (23). Briefly, a silk traction suture or an Ellis clamp is placed at the midpoint of the cartilaginous portion of the recipient airway to retract the bronchus from the mediastinum. The first step is to approximate the donor and recipient posterior peribronchial tissue

followed by a running suture of the membranous portion. The cartilaginous part is sutured with single or figure-of eight stitches, progressively adjusting the mismatch between the stumps and the silk stitch is removed. After completion of the anastomosis, the suture on the posterior peribronchial tissue is continued anteriorly covering the bronchus. This approach has represented, and still represents at several transplant centers, the gold standard. However, some limitations compared to a complete running suture (membranous plus cartilaginous portions) as more time required to perform it, and inflammation caused by multiple stitches that may potentially affect the correct healing have been reported (28,29). For these reasons some authors prefer an end-to-end running technique with an absorbable 4/0 monofilament suture. Although coverage of the anastomosis is usually considered mandatory, at some centers it is not performed at all (Vienna Lung Transplant Center), with equally good results (28). The rate of airway complications is similar with either technique (*Table 1*); furthermore both of them allow easily overcoming of the potential size mismatch between donor and recipient airway.

Telescoping anastomosis with the intussusception of the donor bronchus into the recipient airway has gained widespread consensus in the 1990s to solve the problem of size mismatch and to improve the tightness of anastomosis (30,31); only the cartilaginous part of the bronchus is intussuscepted. However, due to the higher incidence of anastomotic complications, this technique has progressively been relegated to those cases with a natural tendency towards intussusception (28,32).

The technique of bronchial anastomosis in case of pediatric or lobar transplantation (cadaveric or living related) is similar, although the size of the suture is smaller (5/0). In case of living related lobar transplantation, the dissection of the donor bronchus should be minimized to preserve backward blood supply. On right side, the middle lobe bronchus is identified and the incision goes obliquely from above the superior segment bronchus of the lower lobe to just below the middle lobe bronchus; on left side the lower lobe bronchus is transected tangentially above the superior segment of the lower lobe (33).

Risk factors for airway complications after LT

Several risk factors have been considered in the development of airway complications after LT: ischemia, impaired organ preservation, rejection and infection. Prolonged mechanical ventilation of both donor and recipient has been considered to play a role with different mechanisms: by causing a persistent inflammation status and an higher risk of infection in the donor and by determining a barotrauma on the anastomosis in the recipient; furthermore the need of prolonged mechanical ventilation after LT may be a sign of graft failure as a result of prolonged ischemia (25,34). Thus, patients should be extubated as soon as possible (35)

Adequate organ preservation is crucial. The use of low potassium dextrane solutions associated to the administration of prostaglandins to increase the microcirculation flow (36) and the association of retrograde perfusion (37) have contributed to decreased the rate of airway complications; furthermore, limiting the cold ischemic time within 6-8 hours should minimize the risk of injury (38).

Acute rejection has been identified as an independent risk factor for airway complications by causing acute inflammation, submucosal edema and increased vascular resistance with subsequent reduction of graft perfusion (36). Administration of low dose steroids may ameliorate the microcirculation by reducing edema with improvement of perfusion at the anastomotic site; thus, optimizing immunosuppression is crucial.

A strong association between airway complications and Aspergillus infection has been reported (39). Fungal infections are relatively frequent in transplant patients (40). The simultaneous presence of anastomotic necrosis and Aspergillus is correlated with a higher risk of late bronchial complications compared to the presence of necrosis alone. Broncho-arterial fistula has also been reported. An aggressive antifungal therapy should be immediately started even in asymptomatic patients.

Bronchial complications

The incidence of bronchial complications ranges between 7% and 18% with a mortality between 2% and 5% (24,34,41). Early and late complications include bleeding, necrosis, dehiscence, granulations, stenosis and malacia (42). Although several classifications of bronchial healing have been proposed, none has been worldwide accepted. The Couraud grading system based on bronchoscopic surveillance at the 15th postoperative day is well known and it seems to show a correlation with the subsequent onset of airway complications (43). Anastomotic healing is classified as follows:

Grade 1: complete circumferential primary mucosal healing;

Grade 2A: complete circumferential primary healing of

Table 1 Incidence of airway complications after lung transplantation based on the type of bronchial anastomosis

Authors	Year	Type of suture	% of AC
Date	1995	End-to-end interrupted	9.5
Kshettry	1997	Telescoping running[#]	12.4
Herrera	2001	End-to-end running[#]	23.8
Aigner	2003	End-to-end running	2.6
Van De Wauwer	2007	Telescoping interrupted	41.1
Van De Wauwer	2007	End-to-end*	15.7
Weder	2009	End-to-end interrupted	4.9
Van Berkel	2011	End-to-end running standard	8.2
Van Berkel	2011	End-to-end running modified	2.1
Fitzsullivan	2011	End-to-end interrupted standard	18.1
Fitzsullivan	2011	End-to-end interrupted modified	2.3

AC airway complications. [#], nonabsorbable suture; *mixed series with 276 interrupted suture and 28 running suture.

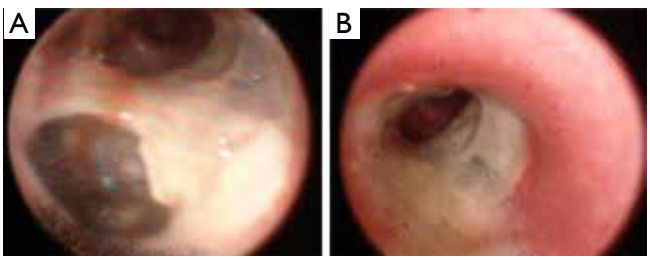

Figure 1 A dehiscence of the bronchial anastomosis can be successfully repaired by careful endoscopic application of fibrin glue. (A) Bronchoscopic view of an anastomotic dehiscence; (B) the dehiscence has been successfully treated with fibrin glue.

the airway wall without necrosis and with partial primary mucosal healing;

Grade 2B: complete circumferential primary healing of the airway wall without necrosis but with no primary mucosal healing;

Grade 3A: limited focal necrosis (extending less than 5 mm from the anastomotic line);

Grade 3B: extensive necrosis.

The development of anastomotic necrosis and dehiscence is related to an ischemic injury and the severity of this complication goes from a focal superficial lesion to extensive necrosis of the bronchial wall that may determine catastrophic consequences with high mortality. These events can be detected in asymptomatic patients during bronchoscopic surveillance or they can be highlighted with radiological studies (computed tomography with multiplanar reconstructions) in patients showing clinical manifestations like fever, cough, dyspnea, prolonged air leaks, pneumothorax, pneumomediastinum with subcutaneous emphysema and mediastinitis with sepsis. When healing, these complications may lead to granulation, stenosis or bronchomalacia. Treatment is based on the severity of the problem, ranging from a conservative approach or minimally invasive treatment for mild lesions to more aggressive therapeutic options including reconstructive surgery, pneumonectomy or retransplantation. In case of a very limited and asymptomatic dehiscence a "wait and see policy" with continuous bronchoscopic surveillance and bronchial debridement can be the first approach; the instillation of glues or sealants has been often reported (44) (*Figure 1*). Stent placement (silicone or covered expandable metallic) is the following step. However, in difficult cases, surgery may be required: direct suture, sleeve resection, pneumonectomy and re- transplantation have been reported, although they carry significant morbidity and mortality (45).

The onset of granuloma usually requires mechanical debridement or laser ablation (46,47). Prevention of recurrence, occurring in 10–50% of cases, includes injection of Anti-fibroblast and anti-inflammatory agents in the bronchial wall to avoid fibroblast proliferation and formation of granulation tissue. Although several drugs have been used, the results are still controversial and the exact dose is not well established yet (47).

The most frequent bronchial complication following LT is stenosis occurring either at the level of the anastomosis or more distally. It is usually related to ischemia and

impaired local microcirculation; however, a diffuse peripheral stricture might be a manifestation of cellular rejection. Patients with bronchial stenosis can be absolutely asymptomatic or present with dyspnea, cough and recurrent pulmonary infections; pulmonary function tests (PFTs) may show a reduction in the forced expiratory volume in 1 second (FEV_1). Treatment includes mechanical dilation with the rigid bronchoscope or other instruments (48), balloon bronchoplasty and stenting. The choice of the stent should be evaluated on a case-by-case basis. Silicone stents are usually easy to deploy, they can be removed even after a long period of time and the cost is low; however, there are some disadvantages including the need of constant nebulization to promote airway clearance and the challenging placement in case of a tortuous airway. In this case the use of metallic stents might be helpful (49); however, the complications related to these devices are formation of granulation tissue, airway rupture due to erosion and extreme difficulty in case removal is required.

Malacia is a condition in which the airway tends to collapse during breathing or with cough and it is generally due to ischemia, infection or altered response of the bronchial wall to immunosuppression. Symptoms are dyspnea and stridor mostly evident during exercise, cough and wheezing; PFTs show a marked reduction of all dynamic volumes [FEV_1, forced expiratory flow of 25% to 75% (FEF_{25-75}) and peak expiratory flow (PEF)]; bronchoscopy allows to confirm the diagnosis. Stenting is usually required.

Overall, early diagnosis of bronchial complications and their correct management are crucial to achieve satisfactory results and a better survival after LT.

Acknowledgements

None.

Footnote

Conflict of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Anile M, Diso D, Rendina EA, Venuta F. Airway anastomosis for lung transplantation. J Thorac Dis 2016;8(Suppl 2):S197-S203. doi: 10.3978/j.issn.2072-1439.2016.01.67

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

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

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

Submitted Jun 24, 2014. Accepted for publication Oct 27, 2014.
doi: 10.3978/j.issn.2072-1439.2014.11.34
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.11.34

Introduction

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

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

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

Lung volume reduction (LVR) practises

Physiological basis for LVR

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

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

Lung volume reduction (LVR) surgery

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

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

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

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

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

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

The sub-groups were:

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

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

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

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

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

Surgical technique and considerations

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

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

Non surgical methods for LVR

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

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

Bronchoscopic interventions can be broadly divided into:

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

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

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

Endobronchial valves

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

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

Results of the initial large randomised trial (VENT study) (23) were not as encouraging as the preliminary studies (24). A total of 321 patients were randomised to Zephr™ endobronchial valve placement or best medical care with a 2:1 ratio. A sham procedure was not undertaken in this study. Patients all had severe airflow obstruction and radiologically heterogeneous emphysema quantified on HRCT chest. Although the study showed statistically significant improvement in the primary outcomes at 6 months (FEV_1 4.3% increase; 6MWT 9 meters improvement) the magnitude of these changes was deemed unlikely to be clinically meaningful (25). Pre-defined major complications were seen in 4.2% of patients undergoing valve therapy. Although not pre-defined as major complications, 7.9% and 5.6% of patients experienced an exacerbation of COPD requiring hospitalisation or haemoptysis respectively.

The European arm of the VENT trial (n=171) was commenced to support slow recruitment in the American study (26). Target recruitment was eventually achieved hence the European cohort being reported separately. Study design was similar to the American arm. When looking at the study population as a whole, a statistically significant improvement at 6 months was seen in only cycle ergometry (5 watts mean improvement compared to controls; $P<0.05$) and SGRQ. The change in SGRQ (5 points) was again below the threshold considered clinically meaningful. The reported focus on this second paper from the VENT group was the effect of collateral ventilation and complete lobar isolation. Subjects in the treatment arm underwent further evaluation with HRCT 6 months post procedure to assess degree of airway occlusion and volume reduction of the targeted lobe. Forty-four subjects in the treatment group of 111 had a complete fissure suggesting the absence of collateral ventilation. A complete fissure conferred reduction in lobar volume by 55% compared with 13% where the fissure was incomplete. Lobar isolation was seen in 48% of patients at 6 months (assessed by HRCT) indicating most patients continued to ventilate the targeted lobe despite the procedure. Combining these two variables (no collateral ventilation; successful technical isolation) yielded the most encouraging results. Improvements in FEV_1, 6MWT and St George's questionnaire were all clinically and statistically significant in this instance.

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

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

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

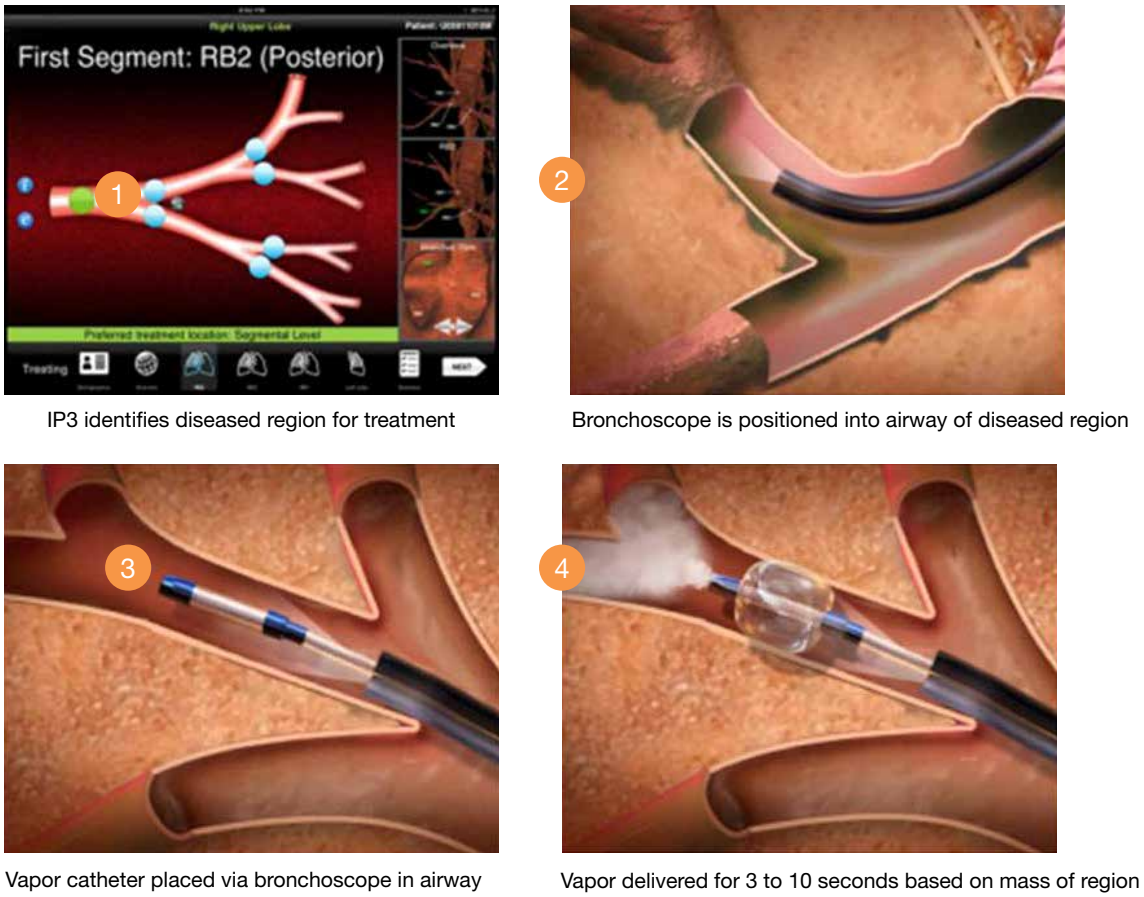

Figure 1 Technical aspects of BTVA-courtesy of uptake medical corporation. BTVA, bronchoscopic thermal vapour ablation.

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

Bronchoscopic thermal vapour ablation (BTVA)

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

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

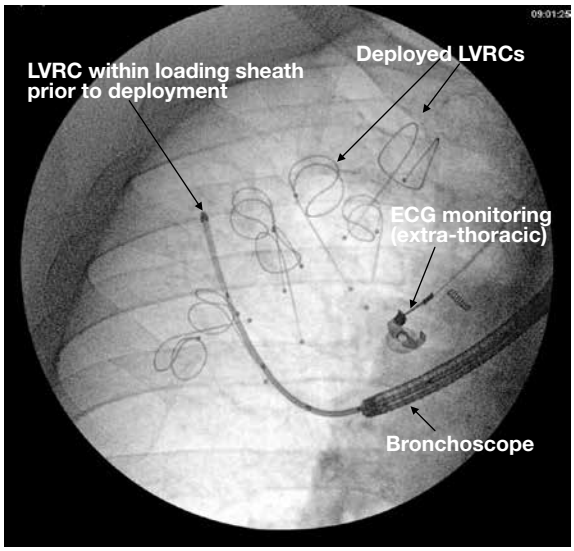

Figure 2 Fluoroscopic appearance of endobronchial coils at bronchoscopy. Seven coils have been sited with the 8th coil remaining within the guide sheath just prior to deployment. Courtesy of PneumoRx Inc.

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

Lung volume reduction coils (LVRCs)

By applying traction forces to lung parenchyma, LVRCs aim to improve hyperinflation and gas trapping by reducing dynamic airway collapse (22). The mechanism of action is again independent of CV and could be applied to emphysema that is homogeneous or heterogeneous (in contrast to BTVA where heterogeneous disease is currently being targeted). The early published data shows promise with larger studies underway (35,36). The technique involves catheterising target lung segments with a guide wire to a distance 3.5 mm to the pleural edge (*Figure 2*). The coil sits within a loading sheath, straightening it prior to deployment. As the sheath and guide wire are withdrawn the LVRC reverts to its prior coiled shape applying traction to the surrounding lung parenchyma. Dynamic expiratory small airway collapse is reduced by application of radial traction thus improving gas trapping and hyperinflation. Up to ten LVRCs can be sited during a procedure initially unilaterally with further scope for a contra-lateral procedure at a later date if tolerated.

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

Biologic lung volume reduction (Bio-LVR)

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

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

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

Endobronchial and extra-pulmonary bypass procedures

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

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

Lung transplantation

Indications for lung transplantation in COPD

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

Patients should ideally be referred to a transplant centre before they are established in the "transplant window"-the time period for which the patient is likely to confer benefit from transplantation prior to becoming too frail to undertake the peri operative rigours and recovery after transplantation. This allows adequate time for assessment, consideration of alternative options (i.e., LVRS as discussed above) and addressing reversible relative contraindications or issues that may impact on the transplant process. Factors

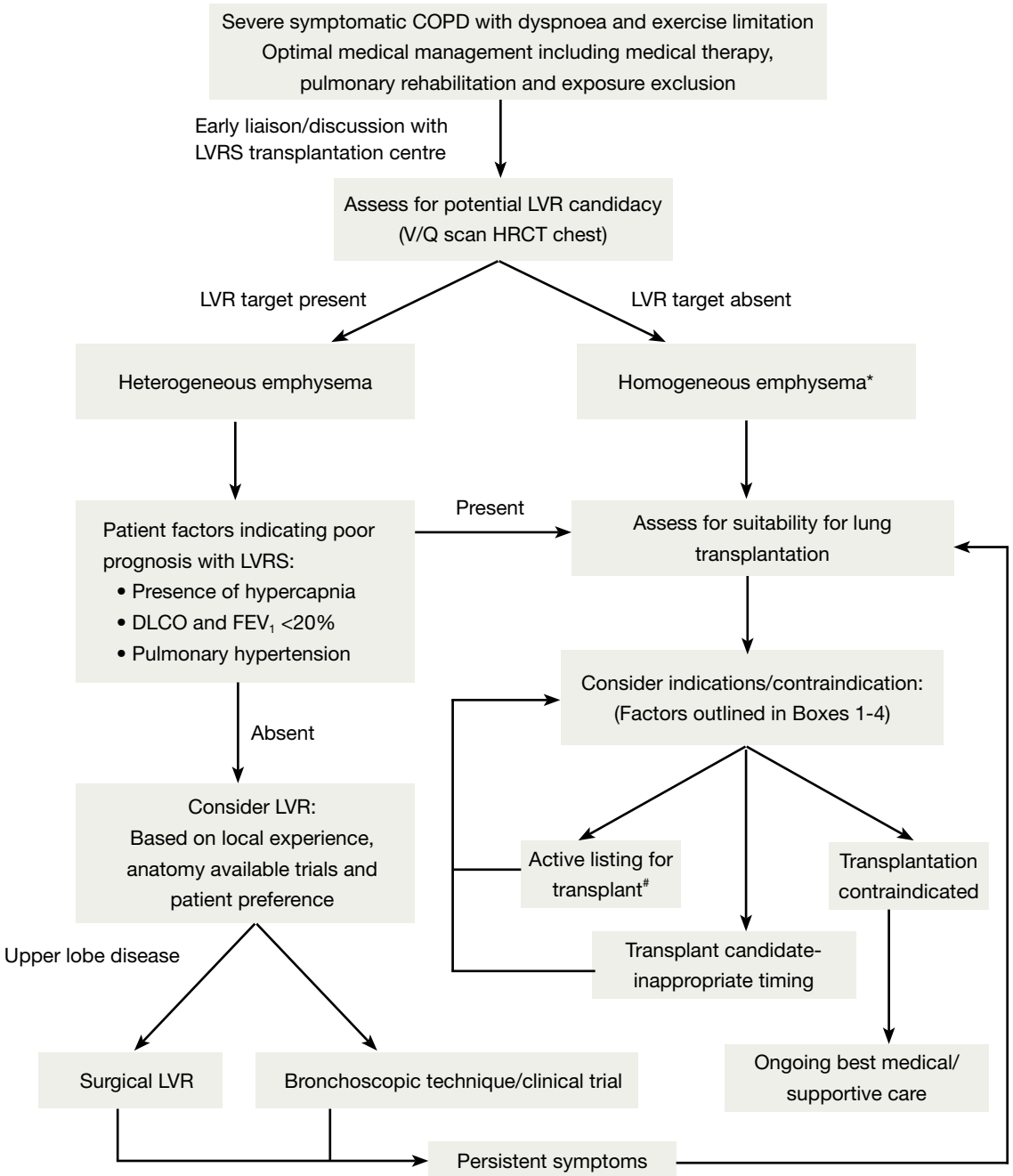

Figure 3 Suggested pathway for management of advanced COPD. *, in selected centres trial may be available for patient with homogenous disease. #, patient active on transplant waiting list require ongoing evaluation. COPD, chronic obstructive pulmonary disease; LVRS, lung volume reduction surgery; LVR, lung volume reduction.

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

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

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

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

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

Scoring systems may also have a role in identifying patients with poor prognosis (50). The BODE score further uses body mass index (B), degree of obstruction (O), dyspnoea (D)-MMRC dyspnoea scale, and exercise capacity (E)-6 minute walk test (6MWT) to stratify which patients have poorer prognosis. Scores of 7-10 confer median survival of 3 years indicating patients are symptomatic, functionally limited and are likely to have a survival benefit from transplantation. The NETT trial also identified a subgroup of patients with poor prognosis. Subjects who did not undergo LVRS (control group) with low FEV_1 (<20%), and either low DLCO (<20%) or homogenous emphysema survived for a median of 3 years although this was significantly better than similar patients undergoing LVRS. Patients with refractory pulmonary hypertension despite oxygen therapy should also be considered given high waiting list mortality (51).

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

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

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

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

Donation after circulatory death (DCD)

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

Table 4 Conventional criterion for acceptance of lung donors
Age <55
PaO_2 >300 mmHg (5 mmHg PEEP FiO_2 100%)
Clear chest X-ray
Less than 20 pack years smoking
Absence of chest trauma
Absence of prior thoracic surgery
Absence of aspiration or sepsis

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

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

Ex vivo lung perfusion (EVLP)

Lung transplantation is dependent on the availability of organs from suitable donors. Respiratory complications in potential lung donors contribute to a low proportion of organs proceeding to transplantation. Common donor mechanisms of death-chest trauma, aspiration, ventilator associated pneumonia, barotrauma and systemic inflammatory response syndrome all impact on organ utility. Transplant physicians exercise caution when assessing potential donor lungs to minimise the risk of morbidity and mortality from PGD-a condition associated with inferior short and long term outcomes (64). It is seen more frequently in patients where there is deviation from traditional donor acceptance criterion (*Table 4*) (65). These parameters will minimise the risk of PGD but lead to a low proportion of potential donors converting to transplant. Of organs offered for transplant a low proportion—15% to 20%—are utilised (66). Strategies to safely increase the number of "marginal" donors-those organs with clinical features/parameters deviating from traditional acceptance-will have an impact on numbers of patients able to undergo transplantation. Reported results from some larger transplant centres suggest those traditional acceptance criterions are overly stringent (67) with transplantation being safely undertaken where the donor does not fully adhere to this criteria. Recognition that these criteria are not absolute may be contributing to recovery of a higher proportion of organs (68). EVLP is a further tool that has potential to further improve this trend.

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

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

As outlined above, procurement of DCD donors has been used as an indication for EVLP (63) although other centres have demonstrates satisfactory DCD outcomes without this additional assessment (58). Controversy exists with regard to EVLP in where it should be employed. As mentioned above, a proportion of marginal donors can be utilised without EVLP assessment without compromising outcomes (67); given this more work is required to redefine the boundaries of donor conventional donor acceptability. Such studies may define where marginal lungs could be utilised without EVLP-without this information there is a

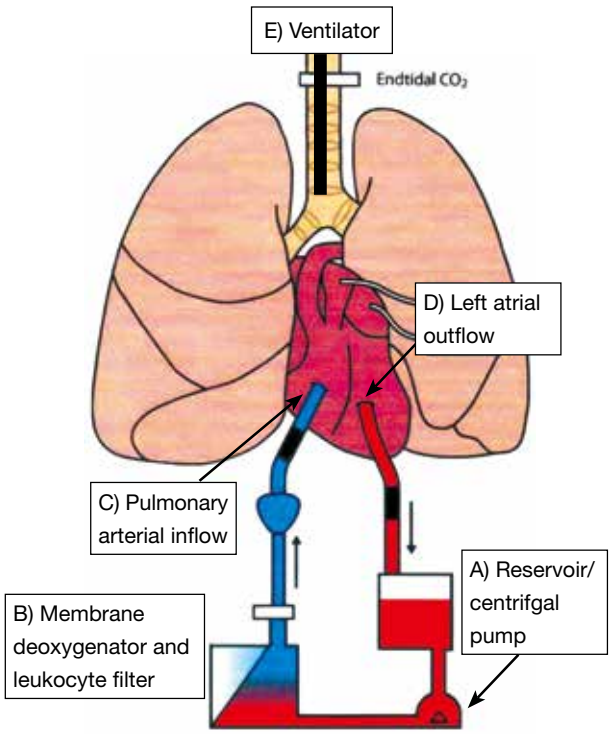

Figure 4 Summary diagram of the EVLP circuit: (A) steen solution™ and blood are circulated via a centrifugal pump; (B) a membrane de-oxygenator allows assumption and regulation of gas pressures equivalent to mixed venous blood. The leukocyte filter minimised leukocyte mediated tissue injury; (C) the pulmonary artery is cannulated. Pulmonary arterial pressure is monitored and flow rate regulated to prevent oedema; (D) left atrial outflow is sampled allowing graft assessment; (E) gentle ventilation commences at a temperature of 32 ℃ full ventilation at 37 ℃ prior to graft assessment. EVLP, ex vivo lung perfusion.

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

The EVLP circuit consists of a sterile chamber housing the donor lungs, centrifugal pump circulating the perfusate, leucocyte filter and membrane de-oxygenator (*Figure 4*). Two differing protocols are currently used and referred to as Lund protocol (72) and Toronto protocol (64), although the general principles are common to the two methods. The perfusate provides above normal oncotic pressure and inhibits endothelial leucocyte interaction, generation of reactive oxygen species and thrombogenesis. Gradual warming of the solution occurs to 37 ℃ allowing restoration of cellular metabolic pathways permitting return to physiological conditions at normothermia. Antibiotics can be administered and interstitial oedema improved via hyperosmolar perfusate mediated fluid shifts. Lungs are connected at an initial perfusate temperature of 15 ℃; at a temperature of 32 ℃ gentle ventilation is commenced with recruitment manoeuvres enabling re-expansion of lobar or segmental collapse. Bronchoscopy may also be performed to assess for and remove secretions from the tracheo-bronchial tree.

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

Conclusions

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

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

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Trotter MA, Hopkins PM. Advanced therapies for COPD—What's on the horizon? Progress in lung volume reduction and lung transplantation. J Thorac Dis 2014;6(11):1640-1653. doi: 10.3978/j.issn.2072-1439.2014.11.34

Pulmonary retransplantation

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Abstract: Lung retransplantation has developed quickly these years with the increasing number of primary lung transplantation. But the operative risk and survival of lung retransplantation was inferior to primary lung transplantation. The bronchiolitis obliterans syndrome (BOS), the early graft failure and the irreversible airway complications are the main causes for lung retransplantation. In this review, we give a general view of the history of lung retransplantation. And we reviewed the factor related to the prognosis of lung retransplantation according to the previous publications. There have been four lung retransplantation in Shanghai Chest Hospital till now. We shared our preliminary experience here.

Keywords: Lung; retransplantation

Submitted Jun 06, 2018. Accepted for publication Jul 18, 2018.
doi: 10.21037/jtd.2018.07.94
View this article at: http://dx.doi.org/10.21037/jtd.2018.07.94

In the last 30 years, pulmonary transplantation has become the standard treatment for many end-stage benign lung diseases. Pulmonary transplantation has developed rapidly in the past decade. According to official data from the International Society of Heart and Lung Transplantation (ISHLT), a total of 1,934 adult pulmonary transplantations were performed worldwide in 2003, and 3,893 were performed in 2013. For patients undergoing initial pulmonary transplantation, the 5-year survival rate reported in the literature is approximately 50%, which was lower than the reported survival rates of 71% in heart transplantation, 70% in kidney transplantation, and 67% in liver transplantation. Graft failure caused by chronic rejection or bronchiolitis obliterans syndrome (BOS) is the major factor affecting long-term survival after pulmonary transplantation. Literature reports indicate that the probability of occurrence of BOS within 5 years after initial pulmonary transplantation is approximately 50%. For severe BOS, pulmonary retransplantation is the only effective means to improve survival. The first pulmonary retransplantation surgery in the world was completed in 1985, and the number of retransplantation cases has increased in recent years along with the number of initial transplantation cases. In general, the risk involved with retransplantation is greater than that of the initial transplantation, and the prognosis is also worse than that of the initial transplantation. Nevertheless, with the development of new surgical techniques and immunological drugs, the outcome of retransplantation has significantly improved in recent years. The 1-year survival rate of pulmonary retransplantation in the 1990s was approximately 47%, and data from the United Network for Organ Sharing (UNOS) database collected during 2004–2013 showed an improvement in survival to approximately 71.1%. Although this is still lower than the survival associated with initial transplantation, it was indeed a significant improvement (1,2). This study will review the current status of retransplantation and describe developments in retransplantation, the rational selection of patients, and the prognosis.

History of pulmonary retransplantation

Before the 1990s, as pulmonary transplantation was

Table 1 Main indications and contraindications currently considered

Indications for pulmonary retransplantation
Age ≤60 years
Delayed graft failure
BOS stage 3
Ambulatory
No ventilator dependence
Controllable bacterial or fungal infections
Compliant with treatment
Contraindications/relative contraindications to pulmonary retransplantation
Age >60 years
BMI <15 or >30
Bedridden
Multiple organ failure
Chronic renal insufficiency
Mechanical ventilatory support dependence
Bacterial infection with pan-drug resistance
Noncompliant with treatment

just in its infancy and all the related technologies were still immature, the number of patients undergoing pulmonary retransplantation was very small. The pulmonary retransplantation registration system was developed by Novick *et al.* in 1995. In 1998, this team analyzed 230 cases of pulmonary retransplantation in 47 transplantation centers, reporting a 1-year survival rate of 47%, significantly lower than the 73% observed for initial transplantation (1). A few studies on retransplantation have been conducted since then. In 2003, Brugiere *et al.* (3) described 15 cases of retransplantation due to BOS; 40% of the patients could not get out of bed before the retransplantation and the postoperative 1-year survival rate was 60%, which again raised expectations for the outcome of retransplantation. With the increase in the cases of initial pulmonary transplantation, retransplantation has also increased rapidly. According to a report by the organ procurement transplantation network (OPTN), pulmonary retransplantation accounted for 2.9% of all pulmonary transplantation in 2003, which increased to 5.3% in 2005. The outcomes of pulmonary retransplantation have continued to improve in recent years. In an analysis of the UNOS registration data, Kawut *et al.* (4) found that the outcomes of retransplantation between 2001 and 2006 were better compared to before 2001. Improvements in modern pulmonary transplantation are likely related to improvements in pulmonary preservation technology and the development of immunosuppressive drugs. The appropriate selection of cases may also have contributed to these improvements. The outcomes of surgery for BOS were significantly better than those of retransplantation for short-term graft failure and irreversible airway complications. Retransplantation for BOS is increasingly favored. Aigner *et al.* at the Vienna Transplantation Center (5) retrospectively analyzed 46 cases of pulmonary retransplantation, including 19 cases of BOS, 23 cases of primary graft failure and four cases of airway complications. The survival rates at 30 days, one year and five years after surgery were 89.2%, 72.5%, and 61.3%, respectively. The survival rate of the BOS group was similar to that of patients undergoing initial pulmonary transplantation. In 2006, the Hannover Transplantation Center (6) reported the outcomes of many cases of lung retransplantation at their center. Of the 614 cases of pulmonary transplantation, 54 involved retransplantation. The 5-year survival rate for pulmonary retransplantation due to BOS was 62%, essentially similar to the 63% 5-year survival rate for initial pulmonary transplantation. In 2008, Biswas *et al.* (7) investigated 29 cases among 419 patients with chronic graft failure undergoing retransplantation and found no significant difference between the 1- and 5-year survival rates of these patients and those of patients undergoing initial transplantation. These reports indicate that the outcomes of pulmonary retransplantation have greatly improved and that chronic graft failure is the most appropriate surgical indication for retransplantation.

Indications and contraindications of pulmonary retransplantation

Currently, the main reasons for retransplantation include early severe primary graft failure, irreversible airway complications (such as anastomosis dehiscence), and end-stage BOS. The main indications and contraindications currently considered are listed in *Table 1*.

Factors associated with the prognosis of pulmonary retransplantation

There are many factors affecting the prognosis of pulmonary retransplantation. The factors that have been identified in the literature include the reason for retransplantation, the

number of cases of transplantation performed each year in the institution, the interval between initial transplantation and retransplantation, the patient's pre-transplantation functional status, the time of retransplantation, the surgical procedure for retransplantation, and the age of the patient at the time of retransplantation. (I) The reason for retransplantation. The prognosis of retransplantation due to acute graft failure and airway dehiscence is poor, whereas the prognosis of retransplantation due to end-stage BOS is better. In a study conducted by Strueber *et al.* (6), out of 614 cases of pulmonary transplantation, a total of 54 involved retransplantation. The authors found that the prognosis of retransplantation due to BOS was better, with a 1-year survival rate of 78% and a 5-year survival rate of 62%, which was not significantly different from the prognosis of initial pulmonary transplantation. However, for patients undergoing retransplantation due to early graft failure and anastomotic dehiscence, and patients requiring mechanical ventilation prior to surgery, the 1-year survival rate after retransplantation was approximately 50%. Thus, the reason for retransplantation is an important factor affecting the prognosis. (II) Number of cases of retransplantation. Novick *et al.* (1) analyzed retransplantation at 35 medical institutions and found that the 2-year survival rates for retransplantation in the institutions that had performed more than five retransplantation cases were significantly higher than those performed in institutions with fewer than five cases. (III) The interval between initial transplantation and retransplantation. Kawut *et al.* (4) found that patients who underwent retransplantation within 30 days of the initial transplantation had a significantly higher mortality rate than those undergoing retransplantation after 30 days or more. In a retrospective multivariate analysis of data on 604 patients who underwent lung retransplantation in 2004–2013, Thomas *et al.* (2) found that patients who underwent retransplantation at more than one year after initial transplantation had a significantly better prognosis than those with a retransplantation interval of less than 1 year. (IV) Preoperative functional status. Novick *et al.* (8) analyzed 139 cases of pulmonary retransplantation in 34 institutions and found that approximately 29% of the patients were ambulatory before surgery (walking with or without assistance for more than 50 m). The results of univariate and multivariate analyses indicated that these patients' prognosis was better than that of the patients who could not get out of bed. (V) The time of retransplantation. In general, with the development of technology and drug research and improvements in the understanding of retransplantation, the outcomes of pulmonary retransplantation have improved over time, and they are better now than they were in the early years of this procedure. Of course, improvements in outcomes are also related to changes in the indications and patient selection for pulmonary transplantation and the increase in the proportion of patients with BOS in pulmonary retransplantation in recent years. In a study by Kawut *et al.* (4), the prognosis of pulmonary retransplantation surgeries performed before 2001 was significantly worse than that of retransplantation surgeries performed in 2001–2006. (VI) The surgical procedure used for retransplantation. In an analysis of 325 retransplantation patients registered in the Organ Acquisition and Transplantation Network database (OPTN), Kon *et al.* (9) found that the prognosis of patients who underwent double lung transplantation and contralateral lung transplantation was significantly better than that of the patients with ipsilateral lung transplantation. In a study by Thomas *et al.* (2), the results of multivariate analyses showed that the prognosis of patients undergoing double lung retransplantation was significantly better than that of the patients undergoing single lung retransplantation. (VII) The age of the patient at retransplantation. Hall *et al.* (10) analyzed the prognosis of 542 patients who underwent pulmonary transplantation, of whom 87 underwent retransplantation. The results showed that the prognosis of recipients aged 50–60 years and older was significantly worse than that of the younger patients. In addition, they found that some donor characteristics may also affect transplant recipients' prognosis. For example, it has been reported that the prognosis of retransplantation is significantly poor when the cause of death of the donor is a cerebrovascular accident. In addition, Novick *et al.* (8) reported that retransplantation involving unmatched CMV status between the donor and recipient had a poor prognosis. Kawut *et al.* (4) reported that the prognosis of patients undergoing retransplantation with lungs from male donors was significantly worse than for patients who had female donors.

Critical issues in retransplantation

Although the outcomes of retransplantation have improved over time, there is still a certain gap in the overall surgical risk and prognosis compared with initial pulmonary transplantation, which may be related to the following factors. (I) The influence of initial transplantation surgery.

Retransplantation patients may have severe pleural adhesions due to the initial transplantation surgery. Their anatomy may also deviate from the norm. After the initial operation, structures are less easily identified, and accidental injury may easily occur. Therefore, there may be an increased risk of surgical complications, such as intraoperative hemorrhage. In addition, when the anastomosis is performed, in order to maintain a good bronchial blood supply and avoid poor blood supply to the anastomosis, the original bronchial anastomosis is usually excised and the proximal segment is anastomosed to the donor bronchus. After the anastomosis is completed, the pericardium, the surrounding pleura, the intercostal muscle, or the omentum are generally wrapped around the anastomotic stoma to increase the chance of healing. (II) Long-term drug effects. The long-term use of immunosuppressants such as FK506 after initial transplantation affects renal function. Renal insufficiency itself is associated with hypertension, osteoporosis, anemia, malnutrition, and neurological disorders, which may lead to adverse events. (III) Immune problems. Retransplantation patients are in a state of chronic immunosuppression due to the long-term use of immunosuppressive agents. It is questionable whether these patients need an initial large induction dose of immunosuppressants. Large doses of immunosuppressive agents greatly increase the risk of infection after retransplantation, and infection is still the primary cause of death in retransplantation. Currently, there is no standard plan for changes in immunosuppression regimens. (IV) Criteria to assess the risk of retransplantation and choose appropriate cases. Although the risk of retransplantation is high and the prognosis is poor, it has been reported in the literature that the prognosis of some patients is equivalent to that of patients undergoing initial pulmonary transplantation (for instance, patients undergoing retransplantation due to BOS). For early post-retransplantation complications such as early graft failure and anastomosis dehiscence, postoperative ventilator support, ECMO support, and a large amount of intravenous drugs are often required. These patients are generally in very poor condition, their systemic function is altered, and the re-anastomosis is often in the edema phase, making healing more difficult and thus increasing the risk of poor surgical outcomes. Therefore, in cases of retransplantation due to early complications, assessment of surgical risk and appropriate patient selection to maximize the value of the organs represent a major challenge.

Pulmonary retransplantation experience at the Shanghai Chest Hospital

Since our hospital started performing pulmonary transplantation in 2003, four retransplantation surgeries have been performed. The indication for all the cases was BOS after initial pulmonary transplantation. All four patients underwent unilateral pulmonary transplantation. One patient had initially undergone single lung transplantation, which was followed by contralateral lung retransplantation. The remaining patients had initially undergone double lung transplantation and underwent retransplantation of a single lung. The ages of the four patients at retransplantation were 39, 44, 49, and 71 years. The intervals between the two lung transplantations were 52, 51, 101, and 78 months, respectively. Two patients had a history of intubation due to preoperative CO_2 retention. No cardiopulmonary bypass was used in any of the cases. The three patients who underwent single-lung retransplantation after double-lung initial transplantation has severe adhesions in the chest cavity. However, the most difficult part of the surgery was exposure of hilar structures, especially for the pulmonary arteries, the bronchus and the pulmonary veins, which required significant care to avoid catastrophic hemorrhage. Of the four patients, two (including the patient who underwent contralateral lung retransplantation) developed anastomotic fistulas. The other two cases were discharged without any complications. In terms of postoperative management, our immunosuppression strategy was similar to that used for initial transplantation, and Simulect (Basiliximab) was used for induction. Afterwards, the triple combination of FK506, mycophenolate mofetil, and prednisone was routinely used. However, we have recently become more aggressive prednisone modulation, mainly due to the relatively high rate of anastomotic complications after retransplantation. The patients who survived the longest after retransplantation have survived for nearly four years and continue to have good outcomes.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Yang J, Pan X, Fu S, Zhao H. Pulmonary retransplantation. J Thorac Dis 2018;10(7):4632-4636. doi: 10.21037/jtd.2018.07.94

Application of ex vivo lung perfusion (EVLP) in lung transplantation

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Abstract: Lung transplantation was the ultimate method to treat the end-stage benign lung diseases. Although the lung transplantation has been increasing quickly in the past 30 years, the shortage of donor lung still limited its development. Ex vivo lung perfusion (EVLP) was a promising technique that could provide the platform to preserve, evaluate and repair the donor lung graft. In this article, we give a general review of the development of EVLP, its clinical application and the animal model. With the increasing experience of clinical EVLP, some pre-identified unsuitable donor lungs have been re-evaluated and accepted for transplantation. EVLP have now been considered to be an effective way to expand the donor pool.

Keywords: *Ex vivo* lung perfusion (EVLP); lung transplantation; donor

Submitted Jun 06, 2018. Accepted for publication Jul 18, 2018.
doi: 10.21037/jtd.2018.07.95
View this article at: http://dx.doi.org/10.21037/jtd.2018.07.95

Lung transplantation is the ultimate effective treatment for benign end-stage lung disease. Dr. Hardy performed the first lung transplant in a human in 1963 (1), and the patient survived 18 days after operation. Approximately 40 lung transplants were performed during the following two decades, but the postoperative survival rate was extremely low. Most patients died of immune rejection, infection, or anastomotic complications. With the clinical application of immunosuppressants such as cyclosporine, the Toronto Lung Transplant Group first successfully performed single-lung transplantation in 1983 (2) and double-lung transplantation in 1986 (3). Since then, lung transplantation has been rapidly progressing. According to the International Society for Heart and Lung Transplantation (ISHLT) registry, 3,893 lung transplants were performed and registered worldwide in 2013, indicating considerable progress in lung transplantation. Currently, donor shortage remains a major limitation of lung transplantation. The utilization rate of donor lungs is significantly lower than that of other solid organs. Studies (4) have shown that only 15–20% of donor lungs are effectively utilized, while the rate is 30% for donor hearts. The main reasons for this low rate are related to complications such as pulmonary contusion, aspiration, mechanical ventilation pressure injury, ventilator-associated pneumonia, and neurogenic pulmonary edema. However, according to the reports, approximately 40% of discarded donor lungs are still usable (5,6). In recent years, the number of patients waiting for a lung transplant has been increasing annually, while the death rate among those on the waiting list has also increased because of the donor shortage. Several measures that expand the availability of donor lungs have been used in clinical practice, such as the use of marginal donors, donors of cardiac death, lobar donors for recipients with a small thoracic cavity, and extracorporeal membrane oxygenation (ECMO) support for donor lungs with compromised quality. Ex vivo lung perfusion (EVLP) is a technique used to evaluate and screen compromised donor lungs with potential for recovery. This technique has been already used in lung transplantation centers in North America and Europe.

Brief history of the development of EVLP

Carrel and Lindbergh first proposed the idea of *ex vivo* organ perfusion in 1935 (7). They removed the thyroids of cats and rabbits for *ex vivo* perfusion for approximately one week. EVLP was initially proposed by Hardesty (8), but the idea was abandoned because of unsatisfactory results. In the 1990s, Steen *et al.* (9,10) evaluated lung functions with EVLP and then later reported the preliminary results of a series of pivotal cases of EVLP; in 2000, EVLP was used to evaluate a lung from a non-heart-beating donor before lung transplantation; in 2005, the team performed EVLP to re-evaluate a lung considered ineligible during initial evaluation, and the lung was finally transplanted successfully. These results provided initial experiences for subsequent clinical application of EVLP. In 2006, Wierup *et al.* (11) built upon Steen's experience and conducted a clinical study. The results showed that in six cases, donor lungs were considered ineligible during initial evaluation, re-evaluated and deemed eligible with EVLP, and successfully transplanted. In 2009, the Toronto Lung Transplant Group (12) proposed the Toronto EVLP protocol; in 2011 (13), they published an article in the *New England Journal of Medicine* reporting that in 20 cases, "ineligible" donor lungs were re-evaluated with EVLP and then successfully transplanted. This study showed that using EVLP is feasible in clinical practice.

Mechanism of action and indications for EVLP

EVLP can restore the circulation and ventilation of the *ex vivo* lung. At an ambient temperature of 37 ℃, a membrane oxygenator is used to simulate oxygen consumption in the body via deoxygenation and maintain the physiological state of lungs with specific perfusate and ventilation. The Steen solution is currently the only Food and Drug Administration (FDA)-approved EVLP perfusate for clinical use. The ventilation gas in the lung membrane consists of N_2 (86%), CO_2 (8%), and O_2 (6%). The hypoxic gas mixture removes the oxygen in the circuit to simulate oxygen consumption in the body. The EVLP system includes a ventilator, an endotracheal tube, perfusate and a fluid circuit, a reservoir, an oxygenator, a pump, and a thermostat.

EVLP is currently used mainly to evaluate certain high-risk donor lungs. It is mainly indicated for (13,14): (I) an oxygenation index <300 mmHg; (II) pulmonary edema as indicated by the last chest X-ray; (III) collapse or poor expansion of a donor lung during harvest; (IV) blood transfusion >10 U; and (V) lungs from donors with cardiac death. EVLP is not suitable in cases of apparent pneumonia, severe mechanical lung injury (including multiple lobar injury), or significant aspiration of gastric contents.

After EVLP, a donor lung is considered eligible for transplant if (13,14) the oxygenation index reaches 400 mmHg after 4–6 hours of EVLP; chest X-ray findings are stable or improved; and pulmonary artery pressure, airway pressure, and lung compliance are stable or improved. A reconditioned donor lung is considered ineligible for transplant if the oxygenation index is <400 mmHg; pulmonary arterial pressure, airway pressure, or lung compliance worsens by ≥15% from baseline; and chest X-ray shows worsening signs.

The primary types of EVLP

Currently, three EVLP systems (*Table 1*) are commercially available for clinical use: the Toronto system, the Lund system, and the Organ Care System (OCS). The Toronto system is the most widely used system. The Lund system is an extension of the original EVLP protocol. The OCS is currently the only portable EVLP system. For both the Toronto and Lund systems, the donor lung is cryopreserved after harvest and during transportation and is connected to the EVLP device for perfusion at ambient temperature after it is delivered to the recipient's hospital. The transportation time is counted towards the cold ischemia time. On the other hand, the OCS allows the donor lung to be immediately connected to the EVLP system for perfusion at ambient temperature after cold perfusion and harvest, thereby reducing the cold ischemia time.

During EVLP and evaluation, the graft can be examined in detail by direct touch, bronchoscopy, and imaging studies to rule out tumors, pulmonary contusion, pulmonary edema, infection, embolism, and interstitial lung disease. Pulmonary function evaluation includes blood gas analysis, hemodynamics, and mechanical ventilation parameters over several hours. During this time, lung tissue specimens and bronchoalveolar lavage fluid can be tested for microbial, molecular, and histomorphological markers, which help determine the quality of a donor lung.

Clinical application of EVLP

Currently, EVLP is mainly used to evaluate the quality of a donor lung at large lung transplant centers in North

Table 1 Comparison of different EVLP systems

Parameter	Lund system	Toronto system	OCS
Perfusion			
Target flow	40% of cardiac output	40% of cardiac output	2–2.5 L/min
Pressure (mmHg)			
Pulmonary artery	Depends on the flow	≤20	≤20
Left atrial pressure	3–5	0 (open)	0 (open)
Perfusate	Steen solution	Steen solution + RBCs (HCT: ~14%)	Steen solution + RBCs (HCT: ~15–20%)
Power pump	Rolling pump	Centrifugal pump	Piston pump
Starting temperature (℃)	32	32	34
Tidal volume (mL/kg)	7	5–7	7
Respiratory rate	7	20	10
PEEP (cmH_2O)	5	5	5–7
FiO_2 (%)	21	50	12
Membrane oxygenator gas flow	Titrated to pulmonary artery PCO_2 34–38 mmHg	Titrated to pulmonary artery PCO_2 34–38 mmHg	–
Temperature (℃)			
Start of ventilation	32	32	32
Start of perfusion	15	25	32
Start of evaluation	37	37	37

EVLP, ex vivo lung perfusion; RBC, red blood cell; HCT, hematocrit; OCS, Organ Care System.

America and Europe. In 2011, the results of the first prospective clinical trial "HELP" (13) showed that 20 of 23 high-risk donor lungs proceeded to transplantation after evaluation; in the control group, 116 lung transplants were performed after conventional standard screening of donor lungs. No significant difference was observed in primary graft failure, the length (days) of postoperative mechanical ventilation, ICU stay (days), hospital stay (days), or 30-day mortality. Aigner *et al.* (15) reported nine cases of double-lung transplantation after EVLP evaluation. No significant difference was observed between these nine cases and the 119 cases in the control group (conventional method) in mechanical ventilation, ICU stay (days), hospital stay (days), or 30-day mortality. In the FDA-approved multi-center Novel lung trial, 42 of 76 donor lungs ultimately proceeded to transplantation after EVLP evaluation. No significant difference was observed between these cases and the 42 cases in the control group (conventional method) in early outcomes or 1-year survival. Fisher *et al.* (16) reported the preliminary results of the DEVELOP-UK trial, a non-randomized trial that investigated transplant outcomes of expanded versus standard donors. The results showed that 18 of 53 (34%) lungs from expanded donors were transplanted after EVLP evaluation, with a slightly lower 1-year survival rate than that for lungs from standard donors (n=184), although the difference did not reach statistical significance. Moreover, early graft injury and unscheduled ECMO support rates and medical expenses were higher in the EVLP group. The OCS is a portable EVLP device that allows immediate lung perfusion at ambient temperature after harvest, thereby minimizing the cold ischemia time. Luc *et al.* (17) conducted a phase III study in 151 cases using the OCS versus 169 cases in the control group. The results showed that the 30-day survival rate was 95.7% versus 100%, the 12-month survival rate was 89.4% versus 88.1%, and 72-hour primary graft failure rate was 17.7% versus 29.7%, respectively (P=0.015). The investigators concluded that EVLP reduced 72-hour primary graft failure and may accelerate postoperative recovery and extend long-term survival.

Animal studies on EVLP

Large animals

Advantages: (I) larger size and weight. Large animals such as pigs are advantageous for EVLP studies as experimental parameters, after modification, can be directly applied to human subjects. The size and weight of pigs are similar to those of humans; therefore, the pig is an effective animal model for human conditions. For example, proper tidal volume, positive end-expiratory pressure (PEEP), and perfusion time settings can be used as a basis for clinical trials. In addition, device design and perfusate volume in porcine studies can be used directly in clinical trials, which is not feasible with small animals; (II) similar immune system and physiological environment. The genetic sequence and physiological environment of pigs are closer to those of humans and can better simulate human conditions compared to rats and mice; therefore, the porcine model is an ideal animal model for pre-clinical studies.

Disadvantages: large animals have certain disadvantages, such as being expensive, time-consuming, and labor-intensive, especially with regard to experimental equipment, the perfusate volume required, and labor costs associated with surgical procedures, anesthesia, and experimental management, thus complicating repeat experiments with large animals for validation.

Small animals

Advantages: EVLP studies have been conducted in small animals such as rats, mice, guinea pigs, and rabbits. The experiments are less expensive than those with large animals, which is the greatest advantage of using small animals. In addition, the experiment can be performed by one person, saving time and labor and facilitating repeat experiments as needed for validation. Of the four animals mentioned above, mice are the smallest and most difficult to operate on, while the other three small animals can be operated on without a microscope. Currently, the success rate of lung transplantation in rats is approximately 95%. Fewer studies have been conducted in rabbits and guinea pigs, and most studies involve the ischemia-reperfusion model. Mice are the most difficult to operate on, with a high incidence of accidental injury, but they are a more valuable research tool than rats because of the availability of a large number of protein antibodies and gene probes.

Disadvantages: small animals have certain disadvantages. Rats and mice have a shorter perfusion time. Studies have shown that the perfusion time is 15 minutes to several hours in rats depending on many factors. Studies of lung injury models show that the degree of lung injury after ventilation/perfusion for 15 minutes in rats is similar to that after 4–24 hours in pigs/humans. Moreover, rodent studies have shown a high incidence of pulmonary atelectasis, which, along with airway fluid after lung recruitment, can damage the alveolar epithelium (18,19). In studies with large animals, the cause of atelectasis can be identified, and airway fluid can be removed via bronchoscopy.

Perfusion technique in animal studies

(I) Perfusate: the Steen solution is commercially available and the most commonly used perfusate. Studies have shown that highly permeable and albumin-containing perfusate is best. Cell-free perfusate does not introduce foreign antigens, eliminating the possibility of red blood cell (RBC) dissolution during perfusion. Moreover, perfusate provides various basic substances for lung metabolism. The lung itself can provide oxygenation, and thus perfusate can be oxygen-free, although it must contain glucose and various electrolytes;

(II) Ventilation parameters: during EVLP, mechanical ventilation should be protective in nature. For large animals, the tidal volume is usually 4–6 mL/kg and may be as high as 10 mL/kg; ~4 mL/kg is usually used in rats, and significant lung injury occurs at 10 mL/kg;

(III) Temperature: in general, the temperature should slowly recover during perfusion, usually to 37 ℃ over approximately 30 minutes;

(IV) Perfusion time: studies have shown that the EVLP time is 30 minutes to 3 hours in small animals and up to 14 hours in pigs. EVLP time is related to perfusion settings and animal models;

(V) Pulmonary arterial perfusion flow and pressure: the goal of pulmonary arterial perfusion flow is to reach a certain level of pulmonary artery pressure or pulmonary vascular resistance. Typically, perfusion flow is incrementally increased to the target flow during the first 15–30 minutes of perfusion. The target flow is 40% of the cardiac output for large animals and humans and ~20% for small animals (20). Pulmonary vascular resistance is calculated with pulmonary artery pressure and flow. If lung function is normal during perfusion, then pulmonary vascular resistance will gradually decrease during perfusion,

and increased resistance indicates progressively impaired oxygenation.

The main role of EVLP animal models

Although EVLP technology has been introduced in clinical practice, further experimental studies still rely on dependable animal models.

(I) Organ function evaluation: during EVLP, the physiological function of the lung can be monitored, including pulmonary artery flow, pulmonary artery pressure, pulmonary vascular resistance, the perfusate oxygen content before and after perfusion. These are effective indicators of lung function;

(II) The experimental platform for lung injury: EVLP controls ventilation, allowing the use of animal models for ventilator-associated lung injury, the mechanism of which can be studied by accurately adjusting parameters such as tidal volume and PEEP. Many animal models of acute lung injury *in vivo*, such as loss of surfactant activity, gastric acid-induced lung injury, and lipopolysaccharide (LPS)-induced lung injury, can be used in EVLP models;

(III) Experimental recovery platform and the route of drug administration: first, the perfusate is modified and optimized to identify optimal conditions and parameters for preserving a donor lung. In addition, more routes of drug administration are used during EVLP such that drugs may be added to the perfusate or mechanical ventilation gas to allow a donor lung to recover. This is an important direction for future studies on donor lung recovery. Cosgun *et al.* (21) conducted a study in pigs and added trimetazidine to the perfusate in the experimental group. The results showed that after 4 hours of EVLP, oxygenation indicators were superior in the experimental group compared to those in the control group. Francioli *et al.* (22) added the antioxidant pyrrolidine dithiocarbamate to the perfusate and found that NF-KB was significantly inhibited, pulmonary edema and protein levels in bronchoalveolar lavage fluid were significantly reduced, and TNF-a and IL-6 levels were significantly lower in the experimental group. Currently, most studies on EVLP lung recovery involve adding drugs to the perfusate, which allows the drugs to directly contact the lung during EVLP, thereby enabling better lung recovery. Hijiya *et al.* (23) conducted a study in dogs with cardiac death. During EVLP of donor lungs, a high-concentration, short-acting β-2 agonist was inhaled 4 times during ventilation. The results showed that oxygenation, lung compliance, pulmonary vascular resistance, and pulmonary edema were significantly better in the experimental group than those in the control group. These animal studies provide a reference for good study designs for future studies on donor lung recovery. EVLP can be used to recover and evaluate donor lungs before transplantation, minimizing adverse effects on the recipient's quality of life associated with transplantation of an ineligible donor lung.

EVLP is an emerging, revolutionary technology that plays a very important role in donor lung recovery and evaluation. It also helps expand the availability of donor lungs and alleviates the shortage of lung transplant donors.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Pan X, Yang J, Fu S, Zhao H. Application of *ex vivo* lung perfusion (EVLP) in lung transplantation. J Thorac Dis 2018;10(7):4637-4642. doi: 10.21037/jtd.2018.07.95

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Updated on October 11, 2018