Lung transplantation: improving clinical outcome and donor organ scarcity

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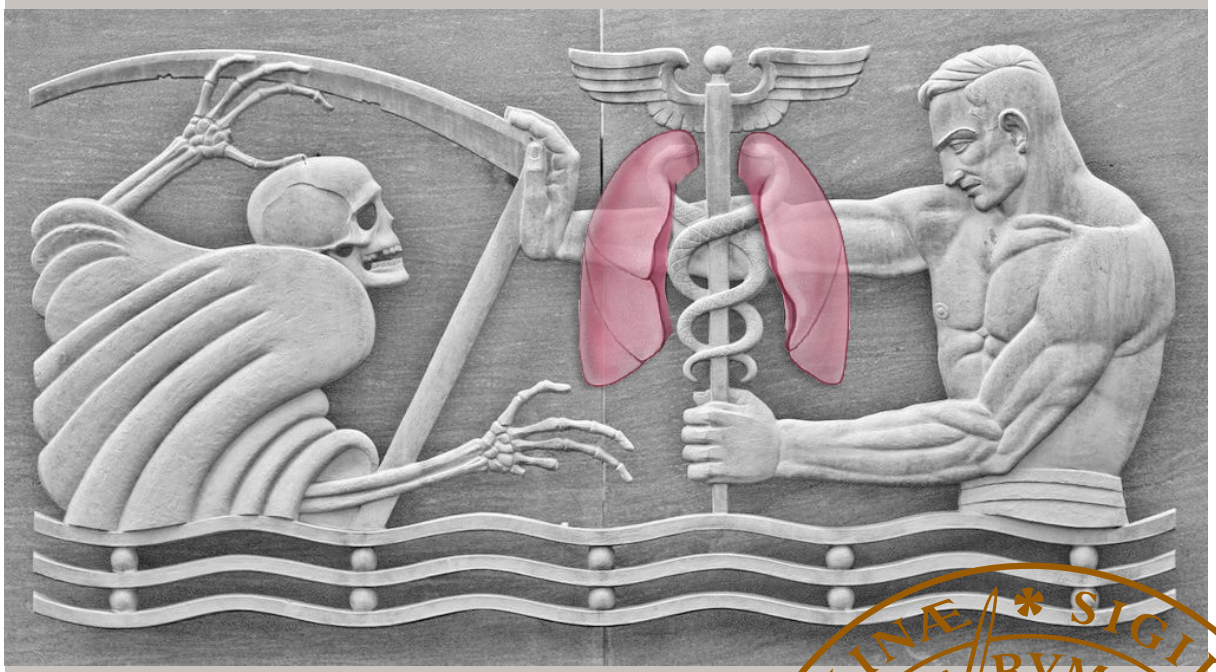

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Mohammed Fakhro, MD

LUND
UNIVERSITY

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Abstract

Background

Despite lung transplantation (LTx) being the golden standard for treating end-stage irreversible pulmonary disease, long-term outcome is yet to date significantly shorter when compared to survival of other solid organ Txs. Chronic lung allograft dysfunction (CLAD) and subsequently bronchiolitis obliterans syndrome (BOS) remains the major barrier to long-term success after LTx. The aim of this thesis was to identify predisposing factors / risk factors related to CLAD/BOS that influence pulmonary function and survival after lung transplantation. In addition to explore the hypothesis that different pulmonary graft preservation influence the outcomes in LTx.

Methods

This dissertation is based on the retrospective review of patient journals at Lund University Hospital from 1990-2016 and a porcine animal DCD lungtransplant model in the experimental aspect of this thesis.

Results

Overall 1-, 5-, 10-, 15- and 20-year survival rates were 88, 65, 49, 37 and 19% for the whole cohort. Double-LTx (DLTx) had superior outcome compared with Single-LTx (SLTx) up to 20-year survival rates ($p < 0.05$). Cumulative incidence rates of BOS were 15% at 5 years, 26% at 10 years and 32% at 20 years. ABO-identical blood group matching showed no superior outcome to ABO-compatible matching ($p > 0.05$).

In the experimental project, investigated blood gases in Donation after Circulatory Death (DCD) in addition to Ex Vivo Lung Perfusion (EVLP) using alteplase, all meet the criteria's for LTx, with no difference compared to the control group in macroscopic appearance ($p > 0.05$).

Conclusion

With a 25-year experience, excellent survival rates were shown giving our recipients one of the highest survival rates internationally. DLTx showed a superior survival capacity over SLTx, despite having a higher incidence of BOS. The present selection-bias of preferring ABO-identical lungs could be refined and help us implement more efficient organ allocation. Futhermore, DCD is a safe and effective method to expand the donor pool.

Key words: Lung transplantation, survival, outcome, bronchiolitis obliterans syndrome, donor organ scarcity, graft preservation, donation after cardiac death, ex vivo lung perfusion

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*Dedicated to my **family**,*

*"To **save** one **life** is to **save** all of **humanity**"*
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List of Publications

This thesis is based on the following publications (in Arabic numerals):

1. Fakhro M, Ingemansson R, Skog I, Algotsson L, Hansson L, Koul B, Gustafsson R, Wierup P, Lindstedt S. 25-year follow-up after lung transplantation at Lund University Hospital in Sweden: superior results obtained for patients with cystic fibrosis. Interact Cardiovasc Thorac Surg 2016; 23: 65-73.
2. Fakhro M, Broberg E, Algotsson L, Hansson L, Koul B, Gustafsson R, Wierup P, Ingemansson R, Lindstedt S. Double lung, unlike single lung transplantation might provide a protective effect on mortality and bronchiolitis obliterans syndrome. J Cardiothorac Surg 2017; 12: 100.
3. Fakhro M, Ingemansson R, Algotsson L, Lindstedt S. Impact of Forced Expiratory Volume in 1 Second (FEV1) and 6-Minute Walking Distance at 3, 6, and 12 Months and Annually on Survival and Occurrence of Bronchiolitis Obliterans Syndrome (BOS) After Lung Transplantation. Ann Transplant 2017; 22: 532-540.
4. Fakhro M, Larsson H, Malmsjo M, Algotsson L, Lindstedt S. ABO-identical matching has no superiority in long-term survival in comparison to ABO-compatible matching in lung transplantation. J Cardiothorac Surg 2019; 14: 24.
5. Liersch-Nordqvist A, Fakhro M, Pierre L, Hlebowicz J, Malmsjö M, Ingemansson R, Lindstedt S. The impact of alteplase on pulmonary graft function in donation after circulatory death - An experimental study. Ann Med Surg (Lond) 2017; 22: 1-6.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Lungtransplantation (LTx) är idag en väletablerad medicinsk intervention för patienter med svår progredierande lungsjukdom, utan möjlighet till något annat behandlingsalternativ. I sådana särskilda fall görs en så kallad LTx utredning och om man uppfyller kraven tas man in på väntelistan för en LTx. Ett av kriterierna är att man har en förväntad livslängd som understiger 2 år utan transplantation. Dock överstiger behovet av donerade lungor tillgången varav många patienter som väntar på lungor aldrig får chansen till ett nytt liv. De vanligaste grundsjukdomarna som orsak till LTx är KOL relaterat emfysem, emfysem relaterat till alfa-1-antitrypsinbrist (AAT1), lungfibros, cystisk fibros (CF) och pulmonell arteriell hypertension (PAH). När man utför en LTx kan man byta båda lungorna i en så kallad dubbellungtransplantation (DLTx), men i vissa fall byter man bara en lunga i en s.k. singellungtransplantation (SLTx) och hos enstaka patienter som har samtidig hjärtsjukdom byter man hela hjärt-lung paketet, vilket kallas hjärt-lungtransplantation (HLTx).

Trots att LTx är den gyllene standarden för att behandla dessa svårt lungsjuka patienter är man fortfarande väldigt utsatt även efter en LTx. Utav alla organtransplantationer som tex njure, lever och hjärta är LTx-patienter de som har sämst överlevnad utav alla dessa grupper på längre sikt. Än idag ser man att akut och kronisk avstötning ansvarar för större delen av dödligheten efter LTx. Det största problemet vid LTx är kronisk rejektion och detta är också den största faktorn som sätter gräns för patienternas överlevnad på längre sikt. Kronisk rejektion definierades tidigare som bronchiolitis obliterans syndrome (BOS) och som idag definieras som chronic lung allograft dysfunction (CLAD). CLAD är mycket ovanligt under det första året efter transplantation men studier visar att 45 till 75 % utvecklar CLAD inom de första fem åren. CLAD ger en successivt ökande lungfunktionsnedsättning och är en obotlig process förutom i vissa sällsynta fall. Idag finns det ingen bra etablerad behandling för CLAD, i vissa terminala fall kan retransplantation erbjudas som en sista utväg. CLAD tillsammans med bristen på donatororgan utgör de två största utmaningarna som vi ställs inför idag inom LTx och som vi behöver lösa för att kunna ge livet åter till dessa svårt sjuka patienter.

Denna avhandling är menad att kartlägga de kliniska svårigheter som LTx patienter bemöter i hopp om att förbättra det kliniska utfallet. Även att vidare utreda hur man kan utvidga donatorpoolen för att möjliggöra fler organ inom LTx.

I denna avhandling har man kunnat påvisa att under en 25-års uppföljning av LTx vid Skånes Universitetssjukhus har man kunnat se att långtidsöverlevnad hos DLTx är

signifikant bättre än hos SLTx patienter. Fördelen hos DLTx kan bero på en bättre förmåga gällande återhämtning av lungfunktion. Likaså påvisades det även att sannolikheten för att utveckla BOS är oberoende av vilken typ LTx man fått, dock visade sig att när man väl utvecklat en kronisk avstötning har DLTx recipienter en markant ökad sannolikhet för överlevnad än SLTx mottagare. Man kunde även visa att 1-årsöverlevnaden låg på 88 %, medan 5- och 10- års överlevnad låg på 65 % respektive 49 % med en median överlevnad på 9.8 år. Dessa siffror är i nivå med de bästa internationellt presenterade siffrorna. Gällande diagnosgrupp visade sig att AAT1 patienter, men framförallt CF patienter levde längst efter transplantation med en median på 11.8 respektive 16.2 år medan KOL och lungfibros-patienter uppvisade sämre överlevnad med en median överlevnad på 6.9 respektive 6.8 år. Högre ålder och förekomst av andra sjukdomar såsom hjärtkärl-sjukdomar skulle kunna vara en bidragande faktor i skillnaden i livslängd.

Att bromsa progressen av BOS är en möjlighet under förutsättningen att man hittar den tidigt nog. Detta är möjligt vid uppföljning och analys av en LTx patients lungfunktion som mäts via spirometri och gångtest. Det påvisades att ju bättre en LTx patient presterar vid ett test av lungfunktion, ju mer ökar sannolikheten för överlevnad. Gällande sannolikheten att vara fri från BOS kunde man se för varje liter luft patienten klarar av vid en spirometrisk undersökning, sänker man dödligheten med 45 %. Likaså när man ber patienten utföra ett gångtest att en ökning med 10 % hos en patient motsvarar att man sänker dödligheten med 21 %. Att hitta ett lungfunktionsmönster kan hjälpa oss att förstå hur vi kan förbättra det kliniska utfallet efter transplantation.

En annan viktig aspekt inom LTx och även övriga organ transplantationer är matchningen mellan donator och mottagare som är grundförutsättning för en lyckad transplantation. En sådan förutsättning är matchningen mellan donator och mottagare mellan olika blodgrupper (ABO). Genom åren har man strävat att matcha blodgrupperna identiskt istället för endast matcha de kompatibelt (Tex A-A istället för A-AB). Dock är det oklart med hur mycket man förbättrar utfallet mellan patienter eller om det överhuvudtaget ger en fördel för överlevnad att matcha de så strikt. Vid tidigare studier har man kunnat se blandade resultat med få studier som tittat på hur ABO-matchning påverkat överlevnaden på längre sikt. I denna avhandling visades det sig att recipienter som erhöll ABO-kompatibla icke-identiskt matchade organ hade samma överlevnad som recipienter som fick ABO-identiskt matchade organ. Det nuvarande kriteriet om att föredra identiskt framför kompatibelt matchade organ kan justeras för att öka fördelningen av donatororgan. Det skulle bli möjligt att minska patientens tid på väntelistan inför att få en LTx, då en identiskt matchad LTx har i genomsnitt 80 % längre väntetid än en kompatibel, icke-identiskt matchad LTx.

LTx är som tidigare nämnt hämmat pga av bristen på organ. Ännu ett sätt som dramatiskt skulle kunna öka tillgängligheten av donatororgan är att dra nytta av donation efter cirkulationsstillestånd, sk *donation after circulatory death* (DCD). Hur man bäst bevarar lungor vid DCD och gällande om man behöver använda sig av läkemedel för att förebygga proppar i donatorlungorna vid LTx är fortfarande under diskussion inom fältet. I denna avhandling undersöktes det i en simulerad klinisk DCD situation om tillägget av ett läkemedel som löser upp proppar (Alteplas) vid uttag av donatorlungor skulle förhindra proppar, därav förbättra donatororganets funktion. Alla undersökta lungor i experimentet, både de som blivit behandlade och de som inte blivit behandlade med Alteplas, visade sig ha utmärkt funktion efter att ha blivit evaluerade med hjälp av en metod som kallas för EVLP (Ex-Vivo Lung Perfusion), där samtliga lungor uppnådde kriterierna för klinisk LTx. Dock föreföll inte användningen av Alteplas inte ge någon uppenbar fördelar vid en DCD situation hos donatorlungorna. DCD visade sig vara ett effektivt och säkert sätt att kunna expandera och dra nytta av donor organ som annars ej skulle ha varit till användning.

Sammanfattningsvis har denna avhandling kunnat vidare utreda de tidigare kända utmaningar en LTx patient bemöter inför, under och efter en transplantation med upptäckt av nya fynd som kan förbättra överlevnaden hos dessa svårt utsatta patienter. Genom en nyanserad klinisk uppföljning och med hjälp av experimentellt tillvägagångsätt har denna avhandling tagit fram nya råd och rön för att underlätta de utmaningar LTx patienter står inför genom att maximera antalet transplantationer och förbättra det kliniska utfallet inom LTx.

Abbreviations

6MWT	6-minute walking test
AAT1	Alpha 1-antitrypsin deficiency
ARAD	azithromycin-responsive allograft dysfunction
ATG	anti-thymocyte globulin
BOS	Bronchiolitis Obliterans Syndrome
CF	cystic fibrosis
CLAD	chronic lung allograft dysfunction
COPD	chronic obstructive pulmonary disease
DCD	donation after circulatory death
DLTx	double-lung transplantation
ECMO	extracorporeal membrane oxygenation
EVLP	Ex Vivo Lung Perfusion
FEV1	forced volume expiratory capacity 1 sec
FiO2	inspired oxygen fraction
FVC	forced volume vital capacity
HLTx	heart-lung transplantation
HTx	heart transplantation
ISHLT	the International Society for Heart and Lung Transplantation
LTx	lung transplantation
PaO2	Pre-operative arterial oxygen partial pressure
PAF	pulmonary artery flow
PAP	pulmonary artery pressure
PEEP	positive end-expiratory pressure
PF	pulmonary fibrosis
PGD	primary graft dysfunction
PH	pulmonary hypertension
PVR	pulmonary vascular resistance
Re-LTx	re-lung transplantation
RAS	restrictive allograft syndrome
SLTx	single-lung transplantation

General aims of this dissertation

The overall purpose of this dissertation is to explore the next step in how we may better understand and improve long-term outcome in patients that have underwent lung transplantation (LTx), through clinical follow-up and experimental measures. In essence, how we can improve the long-term survival in LTx patients. It is a highly relevant inquiry as LTx has the worst expected outcome in comparison to other types of solid organ Tx [1]. Such understanding includes mapping risk factors through clinical follow-up such as chronic lung allograft dysfunction (CLAD) with focus on Bronchiolitis Obliterans Syndrome (BOS), pulmonary function, diagnostic groups, LTx type, ABO blood group, CMV/EBV and other important factors that affect the outcome in these patients. In addition, the purpose was to utilize experimental measurers to further solve the grave scarcity of donor lungs hampering potential LTx [2, 3]. This was achieved by studying and optimizing donation after cardiac death (DCD) as well as the tool of assessing and reconditioning donor grafts through Ex Vivo Lung Perfusion (EVLP). These methods are on the cutting edge in the science of LTx, yielding tremendous potential of moving from the experimental setting to the clinical setting, becoming the new golden standard of LTx [4-6].

Improving clinical outcome

In order to set a foundation to this thesis, the first part of the aim was to retrospectively review the entire 25-year experience of the Skåne University Hospital Lung Transplant Program. Particular emphasis was put on both short- and long-term survival but also on different subgroups of patients and type of transplant procedure performed. Achieving this would set the foundation of this dissertation. The next phase included reviewing the entire 25-year experience but instead focusing on another end point such as BOS/CLAD between different subgroups of recipients and type of transplant procedure performed. Moving forward with these sub analyses proved crucial in finding new trends among sub groups of LTx recipients. Establishing certain tendencies among different LTx recipients would allow us to refine the current organ allocation program, allowing for a more individualized patient care and clinical follow up.

Finding pulmonary function trends

As CLAD is one of the most important limitations of long-term survival and quality of life after LTx [7, 8], an important aim would be to investigate the clinical methodologies of finding and monitoring CLAD. Two key methods in the clinical setting is through evaluating pulmonary function such as spirometry (FEV1) and 6-minute walking test (6MWT). Thus, the next step would be to analyze post-transplant pulmonary function using these methods. Comparisons were made between different subgroups of recipients and by type of transplant procedure performed in the hope of finding pulmonary function trends that might help us understand how to improve post-transplantation outcome.

Improving recipient-donor matching

Matching a donor to a potential recipient is of utmost importance before an LTx can take place. Matching factors such as age, height, and size are well-known requirements in an organ allocation program to optimize a successful transplantation and optimal post-operative outcome. Therefore, covering this perspective of LTx in the aim of the dissertation was a necessary objective. One aspect of organ matching between donor-recipient that is yet to date under debate in LTx is ABO blood group matching. ABO-identical matching has long been favored over minor ABO-mismatching (viz. compatible but non-identical) in transplantation, thought to decrease the possibility of organ rejection. Although identical blood group matching among recipient and donor is favored, it is still not determined by how much this improves the outcome for recipients who received an LTx, or whether there is any survival benefit to be had. Therefore, by retrospective cohort study, this dissertation aimed to better understand the impact of ABO-identical vs. ABO-compatible matching on post-transplant survival in LTx and widening the horizon by exploring additional risk factors.

Improving graft preservation

The final aim involved focusing on solving the shortage of donor lungs and waiting-list mortality. In recent years, there has been an increased interest in trying to solve donor organ scarcity by utilizing donation after cardio-circulatory death (DCD). In combination with Ex vivo lung evaluation (EVLP), with the capability of evaluating and reconditioning the donor graft, has shown to be a recognized method for assessing graft function post mortem in DCD settings. However, there are still several challenges in the field of EVLP such as finding the ideal preservation method. One major challenge is the hazard of post circulatory arrest thrombosis in the potential DCD graft,

with the probable progress of ischemia-reperfusion injury. This dissertation aimed to investigate the use of plasminogen activator (alteplase) infusion prior to lung harvesting in a DCD experimental model. The hypothesis would be that dissolving possible thrombi using this method could improve lung quality and performance.

History and previous studies in lung transplantation

The beginning

Over the past 35 years, Lung transplantation (LTx) and heart–lung transplantation (HLTx) has become well-known medical interventions for treating irreversible, end-stage lung diseases in patients in whom ordinary medical options would be inadequate [9]. The first human LTx was accomplished in 1963, with the recipient surviving 18 days before finally succumbing to kidney failure and malnourishment [10]. In spite of the outcome, this confirmed that LTx was practically possible and that rejection could be avoided with immunosuppression, despite being only for a short period. Over the following 15 years, only few LTXs were achieved, as the majority of patients succumbed during the procedure due to anastomotic complications of the bronchs. Nevertheless, in 1981 the first successful HLTx was achieved for a recipient with pulmonary arterial hypertension followed by the first single LTx (SLTx) for pulmonary fibrosis and the first double LTx (DLTx) for emphysema in 1983 and 1986 respectively [11-13]. Over the subsequent years the number of LTx procedures was booming, with the procedure becoming a recognized intervention for end-stage pulmonary disease.

Becoming the golden standard

Survival after LTx are dependent on numerous aspects such as general and organ-specific status of the recipient, the condition of the donor organ and operative technique. The prolonged survival rates attained in the 1980s and 1990s reflect the advancements in graft preservation, operative technique and immunosuppressive agents with the beginning of cyclosporine. Further progress was made regarding recipient and donor matching with improvements made in prophylactic as well as direct treatment of infections in the patient [14]. These developments in averting earlier mortality/morbidity have made it possible to have a broader set of indications for LTx, with a progressive liberalization in selecting donor organs, giving an overall increase in LTx´s, although still limited by graft availability.

Donor organ scarcity

Donor lung scarcity is the main limiting issue to the amount of LTxs that are performed. Graft organ retrieval in LTx have constantly been significantly lower than kidney, liver, and heart Tx. It is estimated that LTx grafts are harvested as low as 15 % of all donors, while kidneys/livers and hearts are harvested at about 88 % and 30 % percent respectively [15]. These discrepancies may be due to the graft susceptibility to possible complications before and after donor brain death that can occur due to aspiration, ventilator affiliated pulmonary injury, pneumonia and pulmonary edema (neurogenic). However, up to 40 % of rejected donor lungs could have been acceptable for LTx [16].

Clinical outcomes

Outcome after LTx can be evaluated using criteria such as survival, life quality, physiologic parameters and cost versus benefit with survival being feasibly the most basic outcome to assess [17, 18]. The international survival rate for when 50 percent of LTx recipients are expected to survive for all adult patients is 6 years with 7.4 years for DLTx patients and 4.6 years for SLTx patients. Still, it is under debate if this survival benefit is linked to the type of LTx or primary patient characteristics. For instance, it has also been suggested regarding the outcome of pulmonary fibrosis (PF) patients that SLTx patients of < 60 years of age had a survival benefit over DLTx patients of the corresponding age [19].

Major indication

Recipient major indication and its influence of post-operative survival has been evaluated [17, 20, 21]. The recipient diagnostic group is more than often age-related. Certain diagnostic groups have been shown to carry higher perioperative risk and risk for primary graft dysfunction. However Chronic Obstructive Pulmonary disease patients (COPD) have been shown to have a better outcome up to 1-year after LTx than Cystic Fibrosis (CF), Pulmonary Hypertension (PH) and alpha-1 antitrypsin deficiency (AAT1).

Causes of death

Primary graft dysfunction and Chronic Lung Allograft Dysfunction

As to the causes of death that a LTx recipient might face, the major cause of death in the first 30 days after LTx is a form of ARDS or diffuse alveolar damage called Primary graft dysfunction (PGD) [17, 22]. As to the long-term cause of death after LTx, Chronic lung allograft dysfunction (CLAD) has been shown to be the major cause [23-25]. The development of CLAD occurs rarely within the first year after LTx with the rate increasing rapidly with a cumulative incidence as high as 40% to 80% up to five years after LTx [26-29]. CLAD that is displayed early after LTx reportedly has been showed to have a worse expected outcome than CLAD that debuts late-onset.

Bronchiolitis obliterans syndrome

Bronchiolitis obliterans (BO) is the most common pathological form of pulmonary injury seen in LTx recipients with advanced loss of pulmonary function. It is thought to be caused by chronic allograft rejection and is distinguished by obliteration of the bronchioles by fibromyxoid granulation tissue. The dispersion is often irregular and of difficulty to find with transbronchial biopsies [24, 26]. Due to BO being problematic to be found in histology, the International Society for Heart and Lung Transplantation (ISHLT) in 1993 founded the criteria for its physiologic analogue, so called bronchiolitis obliterans syndrome (BOS). For the condition to be diagnosed, a permanent 20% drop in the forced expiratory volume in 1 s (FEV1) is required that cannot be derived to any other ongoing pathological progression [30]. As of 2014 the classification for chronic rejection was supplementary extended to CLAD, including a restrictive phenotype called restrictive allograft syndrome (RAS). CLAD is also identified by radiological findings (CT scan) with observable pathological characteristics in the small airways in addition to pulmonary biopsies showing difficult obstruction of the small airways. The introduction of these clinical proxies has permitted new means of measuring the successive impairment of graft function by bronchiolitis obliterans.

Treating BOS

However, in spite of developments in immunosuppressive agents, treating BOS is yet to date challenging. Altering the immunosuppressive regimen is standard in the occurrence of BOS, but the outcome of this is under debate [31, 32]. New tactics has been proposed for example immunosuppressive therapies in aerosolized regimens, as well as exploring more efficient protocols in substituting immunosuppressive agents to delay development of BOS [33, 34]. Macrolide therapy has also been shown as a successful treatment option in CLAD with deterioration of pulmonary capacity being

halted/reversed [35]. The main clinical tools for assessing pulmonary function are by FEV1 and the 6-minute walking test, with post-transplant pulmonary function being of great interest in clinically follow-up and determine the outcome among different LTx recipients.

Finding the perfect match

In order to achieve a successful LTx it is essential to thoroughly match donor and recipient. Such ideal donor criteria include age < 55 years, normal chest x-ray, PaO2/FiO2 over 300 mmHg at 5 PEE, < 20 pack year smoking anamnesis, excluding donor lungs with chest trauma, aspiration or sepsis and no infected exudations or GI contents on shown in bronchoscopy when attaining the grafts. The donor lungs should preferably be excluded if diseased with HIV or other viral infections such as measles, enterovirus, parvovirus or herpetic meningoencephalitis [36-39].

Expanded donor criteria

A majority of the potential donor pool do not meet up with such strict criteria, where expanded donor criteria with exemptions such as abnormal chest radiograph, advanced donor age, low PaO2, types of malignancy, certain forms of donor infection such as bacterial, mycobacterial and viral infections (CMV/EBV, hepatitis B/C) and ABO compatibility.

ABO-compatibility

Regarding ABO-compatibility, ABO-identical matching is favored and may lead to a survival benefit over minor ABO-mismatching (ie non-identical compatible). Nevertheless, there is reported success with ABO-compatible over ABO-identical organ matching [40, 41]. The long history of preferring ABO-identical matching over minor ABO-mismatching may be due to the belief of decreasing graft rejection [42, 43]. Reports examining recipient-donor mismatching of ABO-matching have generated disagreeing data as to the impact on long-term survival in LTx with the long-term benefits being unclear or whether a survival advantage is attained at all. This criterion may needlessly hamper the number of LTxs that can be utilized from an already scarce donor pool [44].

Age and ABO-matching

The interaction between age and ABO-matching might also be vital. An example that has been made known in HTx is that younger recipients have a lesser hazard for post-Tx infections. However, there is a greater hazard for graft rejection when comparing to older recipients [45]. It's been proposed that this association is caused by immunological influences that alters with the recipient age. Nonetheless it is still under debate if ABO-matching interact with patient age in LTx.

Expanding the donor pool

Considering the topic of donor organ scarcity, LTx is yet to date hampered by the lack of donor organs [2, 3]. In the past years there has been a growing demand for Re-LTx which has risen ethical dilemmas on the how to properly allocate donor organs in the already hampered donor pool [46].

DCD and EVLP

The focus on DCD in LTx has rapidly increased over the years due to donor organ scarcity and mortality that is related to waiting for an LTx [47, 48]. In addition, EVLP has earned its place as an excellent method for assessing donor lung function ex vivo in combination with DCD. EVLP is largely applied for evaluating donor lungs from braindead donor at thoracic transplant centers throughout the world, as the intended donor lung(s) was originally turned down for example as a result of non-acceptable blood gases previous to a preliminary LTx [5, 6, 49]. EVLP is the golden standard for assessing whether a donor lung is qualified for an LTx.

Modifying graft preservation

However, when it comes to the most ideal way of preserving the graft and how to treat the donor organ, the use of antithrombotic and fibrinolytic agents are yet to date under discussion. A common practiced method in LTx is to administer intravenous heparin before explantation to circumvent pulmonary thrombosis. In DCD, heparin is required in such a setting to be recirculated. Thus, the dilemma of a DCD setting as a DCD donor per definition must be without circulation at the time of explantation. There is a current ethical debate if it should be allowed to administer heparin to a donor after cardiac death but previous to consent for donation, especially considering the need of

chest compressions for circulating heparin. It has recently been demonstrated that heparin is no longer necessary in DCD-lungs [50].

Plasminogen activator

The hazard of thrombosis after cardiac death in the potential DCD lung with potential damage caused by ischemia-reperfusion has resulted into several methods with mixed results considering fibrinolytic agents. [51, 52]. The investigation of the infusion of plasminogen activator such as alteplase into the perfusion solution at the time of explantation would be of great interest, as preventing thrombosis would yield increased donor organ function and quality.

Methods

Patients

Between January 1990 to June 2014; 278 recipients underwent LTx (Paper I-III) whilst between January 1990 to June 2016; 307 recipients underwent LTx (Paper IV) at Skåne University Hospital in Lund. Up until June 2016, 197 underwent DLTx, 100 SLTx and 10 patients HLTx. The median age of the recipients was 52 (range 12-72 years). As to gender, 145 were males and 162 females. The major diagnostic groups for a LTx/HLTx were COPD (n = 74), CF (n = 59), AAT1 (n = 59), PF (n = 43), PH (n = 39), and a group called "Others" (n =33), including bronchiectasis, sarcoidosis, bronchioalveolary cancer, silicosis, and graft-vs-host disease. Furthermore, 18 recipients had to undergo Re-LTx. Among these, 8 had DLTx and 10 SLTx. As to gender, 12 were male and 6 female. The major diagnostic groups for Re-LTx were CLAD (n = 15), PGD (n = 1), malignancy (n = 1), and mechanical complication (n = 1). A total of 325 Txs (LTx, HLTx, Re-LTx), ABO-identical (n = 262) and ABO-compatible (n = 53) Txs were performed. ABO-compatible LTx recipients composed of group O donors matching for A, B, AB patients along with group A, B donors matching for AB recipients. Circumstances where an ABO compatible donor was utilized rather than an ABO identical Tx were often because of the shortage in donors and the specific evaluation for each patient that the hazard of prolonging the waiting time for a LTx would be too risky.

Table 1:
Indications for transplantation by era

Indication	1990–1993 (n = 22)	1994–1997 (n = 30)	1998–2001 (n = 41)	2002–2005 (n = 59)	2006–2009 (n = 66)	2010–2014 (n = 75)
PF	18% (n = 4)	0% (n = 0)	17% (n = 7)	5% (n = 3)	20% (n = 13)	15% (n = 11)
CF	9% (n = 2)	6% (n = 2)	20% (n = 8)	17% (n = 10)	21% (n = 14)	24% (n = 18)
PH	36% (n = 8)	27% (n = 8)	12% (n = 5)	12% (n = 7)	6% (n = 4)	9% (n = 7)
ReTx	0% (n = 0)	0% (n = 0)	2% (n = 1)	7% (n = 4	5% (n = 3)	9% (n = 7)
COPD	14% (n = 3)	20% (n = 6)	17% (n = 7)	30% (n = 18)	29% (n = 19)	18% (n = 14)
AAT1	23% (n = 5)	30% (n = 9)	20% (n = 8)	22% (n = 13)	13% (n = 9)	15% (n = 11)
Other[a]	0% (n = 0)	17% (n = 5)	12% (n = 5)	7% (n = 4)	6% (n = 4)	10% (n = 7)

[a]Includes bronchiectasis, sarcoidosis, bronchioalveolar cancer, silicosis, ARDS and GVHD.
PF: pulmonary fibrosis; CF: cystic fibrosis; PH: pulmonary hypertension; ReTx: retransplantation; COPD: chronic obstructive pulmonary disease; AAT1: α1-antitrypsin deficiency

Recipient selection

As to recipient selection, patients were prioritized consistent with the the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation [53]. The criteria for including recipients were patients with established chronic pulmonary disease, unresponsive to medical and/or surgical interventions. LTx contenders characteristically were thought to have a survival expectancy of less than 18 months and were dependent on additional O_2 with diminished exercise tolerance. Prospective candidates were often < 65 years, and if older, potential recipients were examined for supplementary comorbidities.

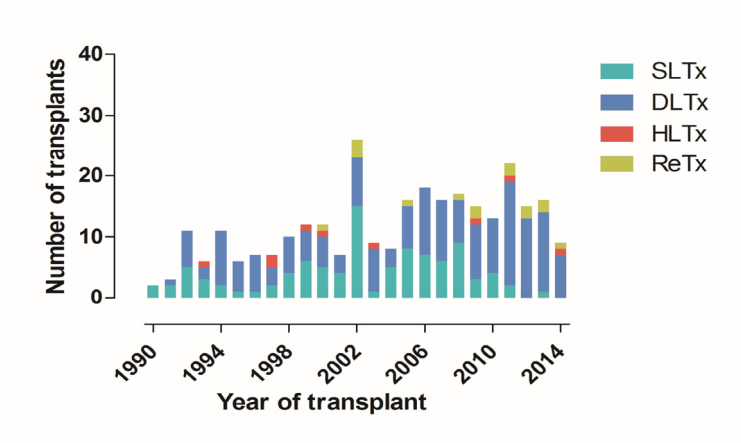

Figure 1: Number and type of transplant performed by year at Skåne University Hospital. HLTx: heart–lung transplant; DLTx: double-lung transplant; SLTx: single-lung transplant; ReTx: re-transplant.

Table 2:
Indications for lung transplantations by type of transplant

Indications	Double lung (n = 179)	Single lung (n = 105)	Heart–lung (n = 9)
Pulmonary hypertension	15% (n = 26)	5% (n = 5)	89% (n = 8)
Cystic fibrosis	29% (n = 52)	1% (n = 1)	11% (n = 1)
Pulmonary fibrosis	11% (n = 20)	17% (n = 18)	0% (n = 0)
COPD	15% (n = 28)	37% (n = 39)	0% (n = 0)
Retransplantation	4% (n = 7)	8% (n = 8)	0% (n = 0)
AAT1	15% (n = 26)	27% (n = 29)	0% (n = 0)
Other[a]	11% (n = 20)	5% (n = 5)	0% (n = 0)

[a]Includes bronchiectasis, sarcoidosis, bronchioalveolar cancer, silicosis, ARDS and GVHD.
GVHD: graft-vs-host disease; COPD: chronic obstructive pulmonary disease; AAT1: α1-antitrypsin deficiency.

Donor organ acquisition

From the LTx programme's beginning, acquired donor organs were perfused in an antegrade manner with EuroCollins solution (<20 mmHg). In 1993, the preservation fluid was changed from EuroCollins to Perfadex (Vitrolife, Göteborg, Sweden). The obtained organs underwent perfusion antegradely with 80 ml/kg of Perfadex® with Addex-THAM (3.3 mmol/ml, Fresenius Kabi AB, Uppsala, Sweden), 2 ml calcium chloride (0.45 mmol/ml) and 3 ml nitroglycerine (5 mg/ml, BMM Pharma AB, Stockholm, Sweden) (<20 mmHg). The Perfadex blend is still utilized yet to date. The lungs were semi-inflated before explantation. Harvested organs were sustained at about 4–8 degrees Celsius.

Chronic lung allograft dysfunction

As to Chronic lung allograft dysfunction, in accordance with the ISHLT guiding principles, BOS was well-defined as > 20% deterioration in FEV1 from the highest acquired baseline [30, 54]. Nevertheless, there is extensive evidence that the histopathology of CLAD is rather miscellaneous, with cellular bronchiolitis recognized in ARAD and pleuroparenchymal fibroelastosis recognized in RAS [54]. Rejection was categorized as acute rejection accompanied by perivascular/interstitial mononuclear cell presence or chronic rejection with bronchiolitis obliterans with thick scarring and eosinophilic cell presence [55]. If swift decline of lung function was spotted as a result of rejection, BOS [56] for instance, bronchoscopies (TBB) were performed to establish a diagnosis and treatment was started by pulsed methylprednisolone (Solu-Medrol, Pfizer AB, Sollentuna, Sweden) as tacrolimus (Prograf, Astellas Pharma AB, Malmö, Sweden) or everolimus (Certican, Novartis AB, Täby, Sweden) replaced cyclosporine. In this dissertation, recipients with BOS grade ≥ 2 was included and chosen for analysis.

Follow-up

Recipients had a comprehensive follow-up and were assessed at consistent intervals of 3, 6, and 12 months and continuing the regimen annually. Spirometry were performed at each follow-up, among others measuring the recipients FEV1 in liters in addition to a 6MWT in expected work percentage.

Ethical aspects

All studies were performed according to the principles of the Helsinki Declaration of Human Rights and were approved by the Regional Ethical Review Board in Lund, Sweden.

The experimental protocol for this study was approved by the Ethics Committee for Animal Research, Lund University, Sweden, Dnr M 172-11. All animals received care according to the European Convention of the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the National Society for Medical Research's Principles of Laboratory Animal Care, and the Institute of Laboratory Animal Research's Guide for the Care and Use of Laboratory Animals.

Clinical cohort analysis

As to the statistical methods for the retrospective cohort research of this thesis, overall survival was established at 1, 3, 5, 10, 15 and 20 years after primary LTx using the Kaplan–Meier method, comparing sets of cohorts using the log-rank test. Recipients were censored reaching the end of the study period or lost to follow-up. Kaplan–Meier survival estimates were shown as percentage survival [95% confidence interval (CI)]. Cox proportional hazards models/competing risk regression analyses were evaluated. Univariate/ univariable models for each risk factor were estimated as well as multivariate/multivariable models. The occurrence of BOS (grade ≥ 2) after primary LTx was analyzed with death acting as competing risk to BOS. In a competing-risks model, we analyzed incidence of BOS grade ≥ 2 and death as two separate outcomes. Comparison between the cumulative incidence of BOS grade ≥ 2 and death was assessed with Gray's test, Gray (1988).

Data are shown as mean (standard deviation), median (range), or frequency (percentage). Shapiro-Wilks test was performed to decide variables that are normally distributed/parametric (mean, SD) versus non-normally distributed/non-parametric (median, range). Independent (unpaired) student's t-test was performed for normally distributed continuous variables opposed to Mann-Whitney U (Wilcoxon rank sum) for non-normally distributed continuous variables. Chi-square/Fisher's exact test were performed for categorical variables. A p-value < 0.05 was determined as statistically significant. SPSS Version 24.0 (IBM Corp.,136 Armonk, NY, USA) was utilized in addition to R with the CMPRSK package (available at http://www.r-project.org).

Experimental cohort analysis

As to the experimental methods of this dissertation, twelve fasting Swedish landrace pigs were utilized and designed as a controlled none blind randomized study (Figure 2).

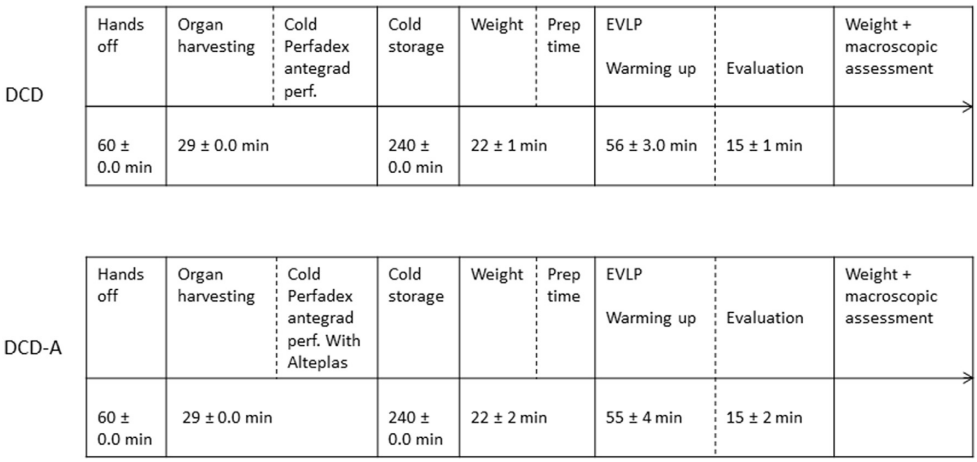

Figure 2: The figure shows a timeline of the experimental setup for the different groups: donation after circulatory death without alteplas (DCD) and with alteplase (DCD-A). The time for each procedural step is given as mean and SEM. In the DCD-A group the lungs were perfused with Perfadex with the addition of alteplase. Ex Vivo Lung Perfusion is mentioned as EVLP in the timeline.

The pigs were randomly assorted into two groups: DCD and DCD with alteplase (DCD-A), with the first group as control. In the DCD group, lungs were perfused antegradely using cold Perfadex with isotonic trometamol, calcium chloride and nitroglycerine. In the DCD-A group, alteplase was added. EVLP was performed and primed with albumin, autologous blood, insulin, imipenem and heparin with isotonic trometamol. Oxygen and CO_2 was supplied to the membrane oxygenator. Low-flow perfusion was started at 25 degrees Celsius through the lungs, gradually warmed by increasing perfusion temperature. Reaching 32 degrees, ventilation was initiated with a FiO_2 of 0.5 with no positive end-expiratory pressure (PEEP). The pump flow was slowly increased and never permitting the pulmonary arterial pressure to surpass 20 mmHg. As normothermia was reached and added PEEP completely expanding the lungs and removing atelectasis with blood gases being analyzed. The lungs were thereby detached from EVLP and put and weighed. The pulmonary arterial branches were macroscopically inspected for thrombosis as the arteries were opened as far distally as possible. As to the calculations and statistics, mean and standard error on the mean (SEM) for various parameters were used for the DCD and DCD-A group. Statistically significant difference for the groups was compared by Mann-Whitney U.

Results

Clinical results

Survival

As to the results of the retrospective analyses of this dissertation, overall survival estimates at 1-, 5-, 10-, 15- and 20-year survival for the entire cohort of patients in terms of percentage of survival were 88%, 65%, 49%, 37% and 19%, respectively (Figure 3).

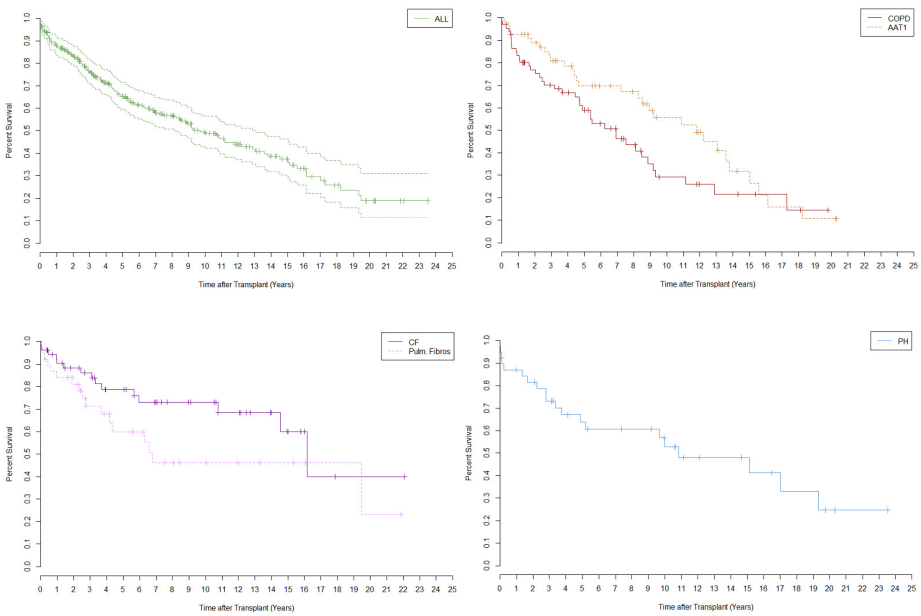

Figure 3: Overall survival after lung transplantation at Skåne University Hospital from January 1990 to June 2014, with a total of 278 patients (top left). Survival for recipients with COPD and AAT1 ($p > 0.05$) (top right). Survival for recipients with CF and PF ($p > 0.05$) (bottom, left). Survival for recipients with PH (bottom right). COPD:chronic obstructive pulmonary disease; AAT1: α1-antitrypsine deficiency; CF: cystic fibrosis; PH: pulmonary hypertension; PF: pulmonary fibrosis.

COPD-recipients had a 1, 5-, 10- and 15-year survival estimates of 83%, 59%, 29% and 22%, correspondingly, comparing with AAT1- recipients at 1-, 5-, 10-, 15- and 20-year survival rates of 93%, 70%, 56%, 32% and 11% ($p > 0.05$). CF-recipients had overall survival estimate of 90% at 1 year, 79% at 5 years, 73% at 10 years, 60% at 15 years and 40% at 20 years comparing with survival estimates in PF recipients at the same time estimates of 84%, 60%, 46%, 46% and 23%, respectively ($p > 0.05$). As to survival by type of transplant, DLTx patients had 1-, 5-, 10- and 20-year survival estimates of 90, 71, 60 and 30%, comparing with SLTx patients with the equivalent survival estimates of 1-, 5-, 10- and 20-year survival rates of 83, 57, 34 and 6% ($p < 0.05$) (Figure 4).

Cause of death

As to causes of mortality, this was investigated in two time windows: >12 and <12 months postoperatively (Table 3). The group called 'other causes' is defined as mortality caused by myocardial and cerebral ischemia, and multiple organ failure in addition to other causes related to the patient's age and health status.

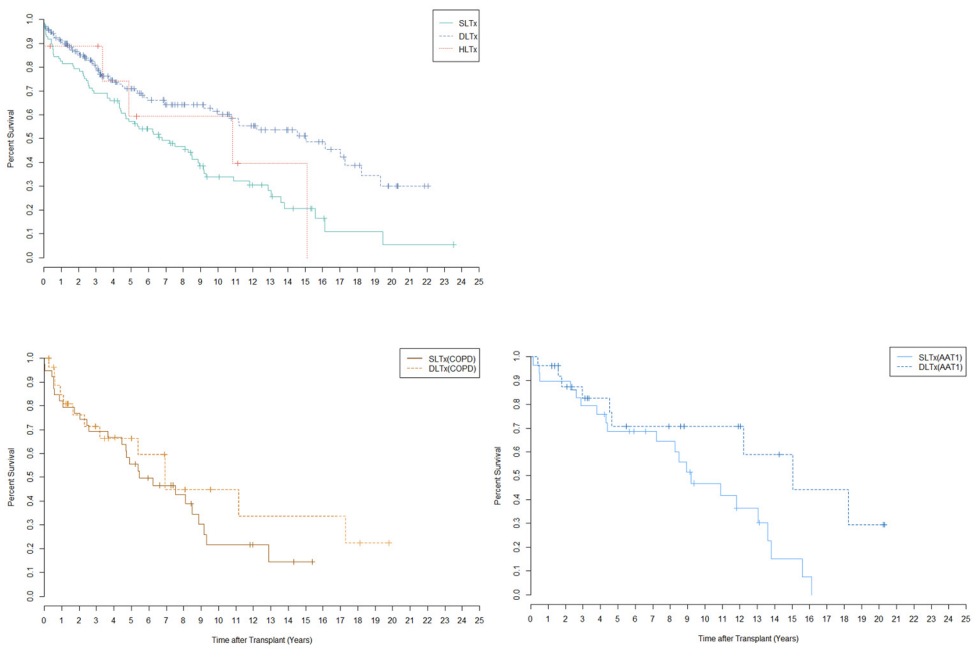

Figure 4: Survival by type of transplant after lung transplantation at Skåne University Hospital from January 1990 to June 2014. HLTx (n = 9), DLTx (n = 172) and SLTx (n = 97) ($p < 0.05$) (top left). Survival in COPD patients by type of transplants, SLTx versus DLTx ($p > 0.05$) (top right). Survival in AAT1 patients by type of transplant, SLTx versus DLTx ($p < 0.05$) (bottom left). COPD: chronic obstructive pulmonary disease; HLTx: heart–lung transplantation; DLTx: double-lung transplantation; SLTx: single-lung transplantation.

Table 3:
Causes of death according to recipient diagnosis and time after transplant

Diagnosis; Cause Of Death	< 12 months	> 12 months	p-value
Total: 278			
Pulmonary fibrosis (n = 38)			
Total number of deaths	6	11	0.903
Death from Organ Rejection	1 (17 %)	2 (18 %)	
Death from Infection	1 (17 %)	4 (36 %)	
Death from Malignancy	2 (33 %)	3 (27 %)	
Death from Other Causes	2 (33 %)	2 (18 %)	
COPD (n = 67)			
Total number of deaths	11	29	0.458
Death from Organ Rejection	4 (36 %)	7 (24 %)	
Death from Infection	2 (18 %)	8 (28 %)	
Death from Malignancy	-	5 (17 %)	
Death from Other Causes	5 (46 %)	9 (31 %)	
AAT1 (n = 55)			
Total number of deaths	4	26	0.718
Death from Organ Rejection	-	6 (23 %)	
Death from Infection	2 (50 %)	7 (27 %)	
Death from Malignancy	-	4 (15 %)	
Death from Other Causes	2 (50 %)	9 (35 %)	
Pulmonary hypertension (n = 39)			
Total number of deaths	5	15	0.208
Death from Organ Rejection	-	5 (33 %)	
Death from Infection	-	2 (13 %)	
Death from Malignancy	-	-	
Death from Other Causes	5 (100 %)	8 (53 %)	
Cystic fibrosis (n = 54)			0.712
Total number of deaths	5	10	
Death from Organ Rejection	1 (20 %)	6 (60 %)	
Death from Infection	2 (40 %)	1 (10 %)	
Death from Malignancy	-	2 (20 %)	
Death from Other Causes	2 (40 %)	1 (10 %)	
Other (n = 25)			0.190
Total number of deaths	3	11	
Death from organ rejection	–	5 (46%)	
Death from infection	1 (33%)	–	
Death from malignancy	–	2 (18%)	
Death from other causes	2 (67%)	4 (36%)	

The group called 'other causes' is defined as patients with mortality caused by myocardial and cerebral ischaemia, and multiple organ failure such as renal and liver in addition to other causes related to the patient's old age and individual health status. COPD: chronic obstructive pulmonary disease; AAT1: α1-antitrypsindeficiency; CF: cystic fibrosis; PF: pulmonary fibrosis

As to ventilator support, intensive care and postoperative stay, overall median time LTx patients had ventilator support after LTx was 1.99 days (0.04–95.00 days) (Table 4). Median length of ICU time was 6.60 days (0.49–105.00 days) in addition to overall median hospital stay after LTx at 42.65 days (11.68–175.66 days). Regarding displaying survival with Cox proportional hazards models (univariable), DLTx patients had HR of 0.514 ($p < 0.05$) compared SLTx patients.

Table 4:
Time of ventilator support, time in the ICU and total hospital time with regard to type of transplant and diagnosis

Indications	Ventilator time	P-value	ICU time	P-value	Hospital time	P-value
LTx type:		0.001		< 0.001		0.386
SLTx	1,0 (0,04-62.0)		4,0 (0,5-81,0)		42,0 (11,7-133,6)	
DLTx	2,0 (0,06-95.0)		7,6 (1,0-105,0)		42,3 (11,8-175,7)	
HLTx	7,0 (1,0-22.0)		57,3 (24,0-91,0)		67,4 (33,0-98,0)	
Diagnosis:		0.007		0.001		0.645
COPD	1,0 (0,04-95.0)		5,2 (0,5-95,2)		44,0 (11,7-163,6)	
AAT1	1,1 (0,22-62,0)		4,0 (0,9-66,0)		42,7 (11,8-175,7)	
CF	1,9 (0,06-29,9)		7,7 (1,0-75,0)		41,4 (11,8-122,0)	
PF	2,6 (0,44-62,0)		8,4 (1,6-81,0)		46,0 (19,9-127,4)	
SPH, EIS	10,5 (1,0-65,5)		36,0 (4,0-69,5)		47,1 (33,0-86,8)	
PPH	4,5 (0,7-80,0)		12,5 (1,0-105,0)		47,0 (23,0-147,0)	
Sarcoidosis	5,8 (0,4-37,0)		19,4 (2,0-73,0)		57,6 (32,0-120,0)	
GVHD	6,3 (1,0-15,1)		11,6 (4,0-22,1)		38,0 (30,0-78,6)	
Bronchiectasis	2,0 (1,3-8,7)		5,9 (3,0-34,7)		41,4 (30,9-63,7)	
Total	2,0 (0,04-95,0)	0.01	6,6 (0,5-105,0)	< 0.001	42,7 (11,67-175,7)	0.386

ICU: intensive care unit; COPD: chronic obstructive pulmonary disease; AAT1: α1-antitrypsin deficiency; CF: cystic fibrosis; PF: pulmonary fibrosis; SPH: secondary pulmonary hypertension; EIS: Eisenmenger's syndrome; PPH: primary pulmonary hypertension; GVHD: graft-vs-host disease.

BOS

As to the incidence of BOS, cumulative incidence of BOS and death for all recipients are shown in Figure 5.

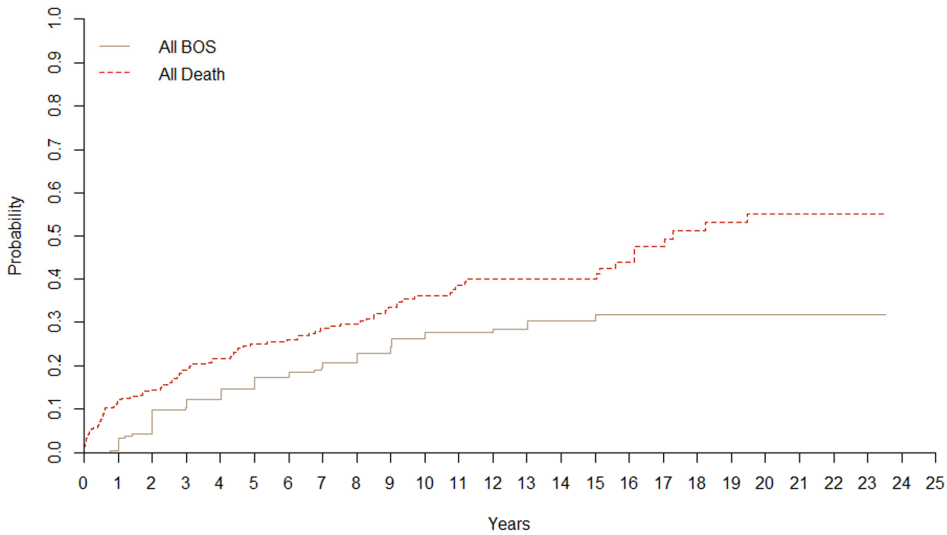

Figure 5: Cumulative incidence of BOS and death, after lung transplantation at Skåne University Hospital from January 1990 to June 2014, for all recipients. BOS: bronchiolitis obliterans syndrome; SLTx: single-lung transplantation; HLTx: heart–lung transplantation; DLTx: double-lung transplantation.

DLTx vs. SLTx

BOS grade ≥ 2 amongst DLTx was 16 ± 3% at 5 years, 30 ± 4% at 10 years, and 37 ± 5% at 20 years when comparing to SLTx with 11 ± 3%, 20 ± 4%, and 24 ± 5% at 5, 10, and 20 years (p > 0. 05) (Figure 6).

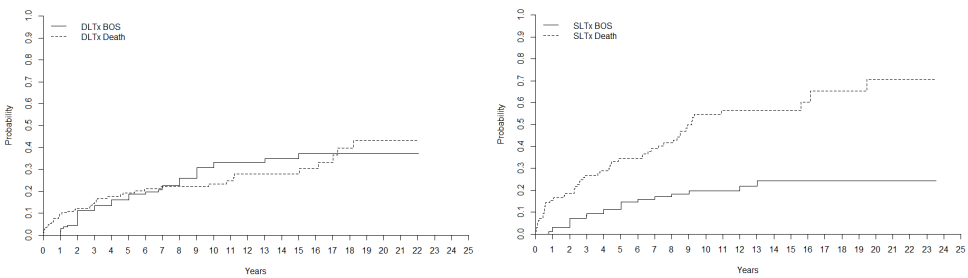

Figure 6: Cumulative incidence of BOS grade ≥ 2 and mortality after LTx in DLTx and SLTx recipients. Note that DLTx and SLTx recipients have the same risk of developing BOS, but DLTx has a significantly better chance of survival despite the presence of BOS

Major indication

By major diagnostic groups, rated in descending order when comparing incidence of BOS grade ≥ 2 was as following: Other, PF, CF, COPD, PH and AAT1 ($p < 0.05$). The mortality estimates by major diagnostic group rated in descending order was COPD, PH, AAT1, PF, Other and CF ($p < 0.05$) (Figure 7).

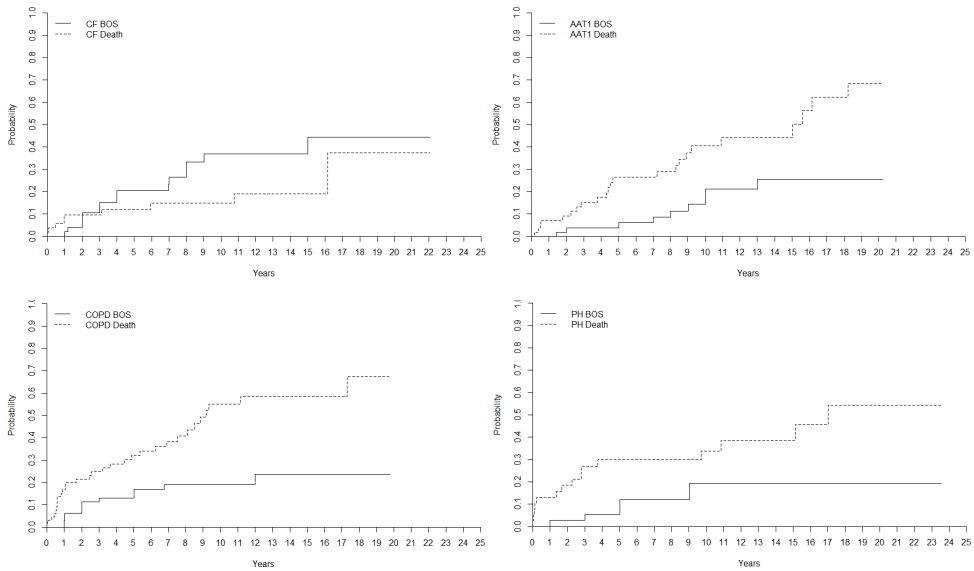

Figure 7: Cumulative incidence of bronchiolitis obliterans syndrome (BOS) and mortality after lung transplantation (LTx) group wise comparing cystic fibrosis (CF), alpha1-antitrypsine deficiency (AAT1) recipients, COPD-recipients and pulmonary hypertension (PH) recipients. CF recipients had a significantly higher risk of developing BOS grade ≥ 2 compared to AAT1 recipients ($p < 0.05$), but AAT1 had a significantly higher mortality ($p < 0.05$), indicating that CF recipients might withstand BOS better than AAT1 recipients. Recipients with CF and COPD had the same incidence of BOS grade ≥ 2 ($p > 0.05$), but chronic obstructive pulmonary disease (COPD) recipients had a significantly higher mortality ($p < 0.05$), indicating that CF recipients might withstand BOS better than COPD recipients. CF recipients had a significantly higher risk of developing BOS grade ≥ 2 compared to PH recipients. However, CF and PH recipients showed the same mortality, indicating that CF and PH recipients with BOS have the same chance of survival

Age and BOS

The incidence of BOS (grade ≥ 2) for recipients ≤50 years of age for recipients >50 years of age is illustrated in figure 8 ($p = 0.238$). The mortality estimated for recipients ≤ 50 years of age was 20 ± 4% at 5 years, 28 ± 4% at 10 years, 34 ± 5% at 15 years and 41 ± 7% at 20 years. For patients >50 years of age the mortality rate was 29 ± 4% at 5 years, 44 ± 5% at 10 years, and 45 ± 5% at 15 years ($p = 0.019$).

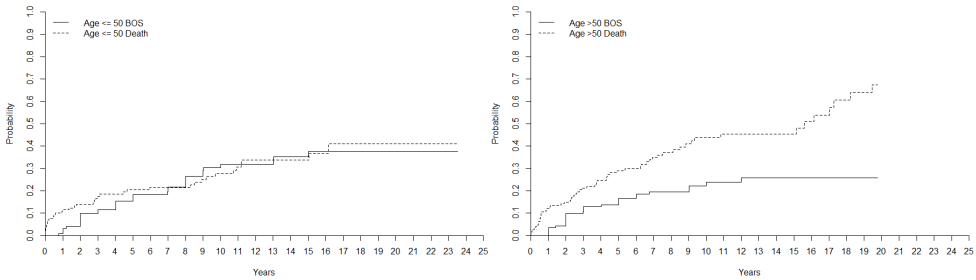

Figure 8: Competing risk analyzing the impact of age on the development of bronchiolitis obliterans syndrome (BOS) and the risk of death after lung transplantation (LTx). Age had no impact on the development of BOS grade ≥ 2, but recipients 50 years or older had a 9% higher mortality 5 years post-transplant and a 16% increased risk 10 years post-transplant compared to recipients younger than 50 years (p < 0. 05)

Time period and BOS

For the time-period 1990 to 2002, the incidence of BOS (grade ≥ 2) in all patients was 9 ± 3% five years, 23 ± 4% at ten years and 29 ± 4 at 20 years. The overall mortality rate for the same time period was 24 ± 4% at five years, 36 ± 4% at ten years and 57 ± 6% at 20 years. Between 2003 to 2014, the incidence of BOS was 8 ± 2% at two years, 21 ± 4% at six years and 29 ± 5% at ten years. The overall mortality rate for the same time period was 14 ± 3% at 2 years and 36 ± 5% at 10 years (Figure 9).

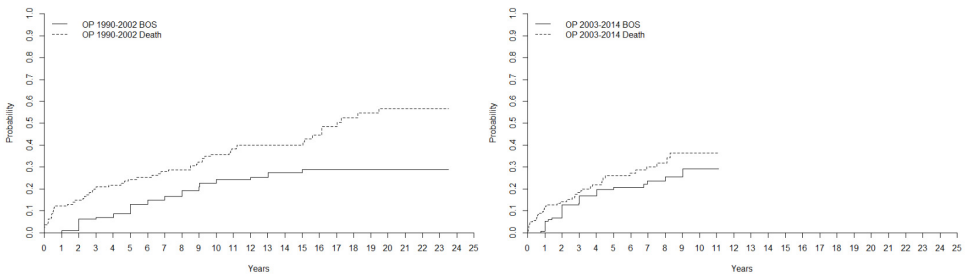

Figure 9: Cumulative incidence of bronchiolitis obliterans syndrome (BOS) and mortality after lung transplantation (LTx) for the two different time periods 1990–2002 and 2003–2014. Our findings (Fig. 1) indicate that DLTx and SLTx carried the same risk of developing BOS grade ≥ 2, but DLTx had a significantly lower risk of death. We suspect that these results might reflect a change in postoperative care towards more aggressive infection and rejection therapy in combination with less frequent SLTx in favor of DLTx the last 10–12 years. However, our results could not confirm these suppositions: no difference was found between the risk of developing BOS grade ≥ 2 or death in different time periods.

Pulmonary function

As to pulmonary function in terms of survival, FEV1 analyses displayed a significant HR of 0.692 (p < 0. 05) in addition to 6MWT analyses with a significant HR of 0.988 (p < 0. 05) (Table 5). As to freedom from BOS (grade ≤ 1) utilizing univariable and multivariable analyses with SLTx patients as reference, FEV1 displayed a significant a

HR of 0.555 (p < 0. 05) whilst 6MWT had a significant result in HR of 0.977 and maintaining significance in the multivariable analyses as well (p < 0. 05) (Table 6). With Tx type in the multivariable freedom from BOS analyses, overall Tx-type illustrated significance (p < 0. 05), with DLTx showing a HR of 1.709 (p < 0. 05) whilst HLTx had a HR 1.759 (p > 0. 05).

Table 5
Cox proportional hazards model evaluating absolute value of baseline FEV1, 6MWT, transplant type and major indications as risk factors for survival

	Hazard ratio	Confidence interval	p-value
Univariable models			
LTx-type			0. 003
DLTx	0. 514	0. 351-0. 752	0. 001
HLTx	0. 888	0. 322-2. 450	0. 818
SLTx (Ref)			
Pulmonary function			
FEV1	0. 692	0. 531-0. 900	0. 006
6MWT	0. 988	0. 977-1. 000	0. 049
Major indication			0. 008
COPD	1. 093	0. 553-2. 159	0. 799
AAT1	0. 728	0. 363-1. 462	0. 373
PH	0. 521	0. 237-1. 143	0. 104
CF	0. 319	0. 138-0. 738	0. 008
PF	0. 668	0. 298-1. 497	0. 327
Age and gender were confounders that were also adjusted for but did not affect the outcome.			

Table 6
Cox proportional hazards model evaluating absolute value of baseline FEV1, 6MWT and transplant type as risk factors for development of freedom from BOS grade ≤ 1

	Hazard ratio	Confidence interval	p-value
Univariable models			
Pulmonary function			
FEV1	0. 555	0. 442-0. 697	<0. 001
6MWT	0. 977	0. 969-0. 985	<0. 001
LTx-type			0. 884
DLTx	0. 940	0. 675-1. 308	0. 714
HLTx	0. 811	0. 293-2. 243	0. 686
SLTx (Ref)			
Multivariable models			
Pulmonary function			
FEV1	0. 597	0. 446-0. 799	<0. 001
6MWT	0. 982	0. 973-0. 991	<0. 001
LTx-type			0. 029
DLTx	1. 709	1. 150-2. 539	0. 008
HLTx	1. 759	0. 533-5. 803	0. 353
SLTx (Ref)			
Confounders such as age and gender were also adjusted for but did not affect the outcome			

Competing risk regression analyses, considering death/Re-LTx and pulmonary function, univariate FEV1 analyses had a regression coefficient of – 0.706 ($p < 0.001$) with 6MWT illustrating a regression coefficient of – 0.026 ($p<0.001$) (Table 7). In the multivariate analyses studying BOS grade ≥ 2, 6MWT demonstrates a regression coefficient of – 0.018 ($p < 0. 05$) and maintaining significance at the multivariate analysis with a regression coefficient of – 0.016 ($p < 0. 05$).

Table 7
Competing risk regression analyses evaluating absolute value of baseline FEV1, 6MWT and LTx-type on the development of BOS grade ≥ 2 and death or Re-LTx: univariate/multivariate models

	BOS			Death / Re-LTx		
	Regression coefficient	Confidence interval	p-value	Regression coefficient	Confidence interval	p-value
Univariate models						
Pulmonary function						
FEV1	+ 0. 208	- 0. 153 to 0. 567	0. 26	- 0. 706	-1. 076 to -0. 336	<0. 001
6MWT	- 0. 007	- 0. 019 to 0. 005	0. 26	- 0. 026	- 0. 038 to -0. 014	<0. 001
LTx-type						
DLTx	+ 0. 373	- 0. 278 to 1. 024	0. 26	-1. 03	- 1. 542 to -0. 518	<0. 001
HLTx	+1. 047	- 0. 662 to 2. 756	0. 23	- 0. 51	- 1. 515 to 0. 495	0.32
SLTx (Ref)						
Multivariate models						
Pulmonary function						
FEV1	+ 0. 361	- 0. 159 to 0. 881	0. 17	- 0. 305	- 0. 771 to 0. 161	0. 2
6MWT	- 0. 018	- 0. 034 to -0. 002	0. 034	- 0. 0162	- 0. 030 to -0. 002	0. 022
LTx-type						
DLTx	+ 0. 268	- 0. 517 to 1. 053	0. 5		- 1. 227 to 0. 020	0. 058
HLTx	+ 1. 030	- 0. 894 to 2. 954	0. 29	- 0. 603	- 1. 606 to 1. 047	0. 68
SLTx (Ref)						
Confounders such as age and gender were also adjusted for but did not affect the outcome						

ABO-matching

Temporal distribution for ABO-identical versus ABO-compatible LTxs conducted between 1990 and 2016 are shown in Figure 10.

As to recipient and donor characteristics for the entire cohort of LTx recipients, it was found statistically significant considering waiting-list time comparing ABO-compatible and ABO-identical LTx with 49 days and 89 days correspondingly ($p < 0.05$) (Table 8).

Cause of death

Causes in mortality regarding follow-up divided into ABO-compatible and ABO-identical LTx is shown in Table 9. No difference was shown in cause of mortality (rejection, infection, malignancy, or "miscellaneous") between ABO-compatible versus ABO-identical groups ($p > 0.05$).

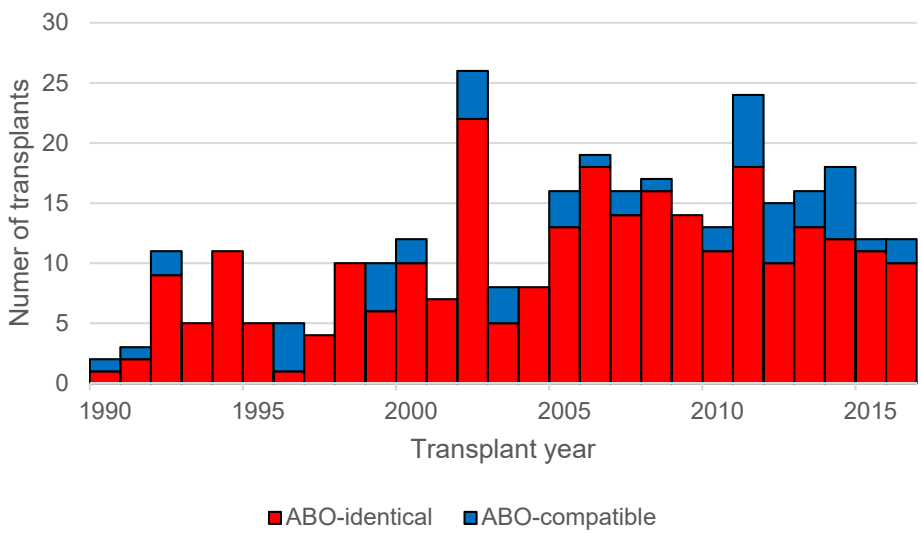

Figure 10: ABO-identical (N = 262) and ABO-compatible (N = 53) transplants between January 1990 to June 2016. Absolute numbers illustrated (bars)

Table 8: Recipient/donor baseline and clinical characteristics of ABO-compatible and ABO-identical LTx

Variables	ABO-compatible (n = 53)	ABO-identical (n = 262)	p-value
Recipient data			
Recipients major indication			0.240
COPD	10 (18.9%)	64 (24.4%)	
AAT1	9 (17.0%)	49 (18.7%)	
PH	7 (13.2%)	29 (11.1%)	
CF	15 (28.3%)	43 (16.4%)	
PF	9 (17.0%)	35 (13.4%)	
Others	2 (3.8%)	25 (9.5%)	
Graft failure (Re-LTx)	1 (1.8%)	17 (6.5%)	
CMV serology (pos)	39 (73.6%)	205 (78.2%)	0. 573
EBV serology (pos)	37 (69.8%)	184 (70.2%)	0. 920
Toxoplasma serology (pos)	14 (26.4%)	62 (23.7%)	0. 680
CMV-mismatch	8 (15.1%)	41 (15.6%)	0. 911
EBV-mismatch	5 (9.4%)	17 (6.5%)	0. 448
Toxoplasma mismatch	6 (11.3%)	31 (11.8%)	0. 916
Weight (kg)	64. 3 ± 19. 9	59. 8 ± 12. 5	0. 032
Recipient/Donor weight ratio	0. 9 (0. 4 - 3. 1)	0. 8 (0. 4 - 1. 6)	0. 034
Height (cm)	169. 1 ± 9. 1	168. 6 ± 10. 4	0. 703
Recipient/Donor height ratio	1. 0 (0. 9 - 2. 4)	0. 9 (0. 8 - 1. 1)	0. 692
BMI	22. 2 ± 4. 2	20. 9 ± 3. 7	0. 045
Male	28 (52.8%)	124 (47.3%)	0. 465
Gender mismatch	16 (30.2%)	90 (34.4%)	0. 523
Age (years)	45. 5 (12. 2 - 70. 6)	52. 9 (12. 4 - 72. 0)	0. 159
Recipient/Donor age ratio	0. 97 (0. 30 - 3. 92)	1. 03 (0. 27-3. 99)	0. 102
Waiting list (days)	49.0 (2. 0 - 641. 0)	89. 0 (1. 0 - 1717. 0)	0. 048
Lab values			
FVC (liters)	2. 0 (0. 7- 5. 2)	2. 1 (0. 3 - 5. 3)	0. 233
FEV1 (liters)	0. 9 (0. 2 - 2. 6)	0. 8 (0. 2 - 3. 4)	0. 735
6MWT (%)	39. 4 ± 20. 3	38. 6 ± 19. 4	0. 813
P-ALT (µkat/L)	0. 4 (0. 1 - 9. 7)	0. 4 (0. 1 - 1. 6)	0. 128
P-AST (µkat/L)	0.5 (0. 2 - 10. 0)	0. 4 (0. 2 - 2. 3)	0. 340
P-creatinine (µmol/L)	66 (22 - 234)	62 (26 - 217)	0. 255
Pulm. pressure > 25mmhg	20 (37.8%)	70 (26.7%)	0. 110
Tx-type			0. 121
SLTx	14 (26.5%)	86 (32.8%)	
DLTx	36 (67.9%)	157 (59.9%)	
HLTx	2 (3.8%)	2 (0.8%)	
Re-LTx	1 (1.8%)	17 (6.5%)	
SLTx	1 (100%)	9 (52.9%)	
DLTx	0 (0%)	8 (47.1%)	
ATG	35 (66.0%)	172 (65.6%)	0. 908
Pre-op Life support			
Mechanical ventilation	2 (3.8%)	12 (4.6%)	0. 795
ECMO	3 (5.7%)	9 (3.4%)	0. 673

Data are mean (SD), number (%), or median (range). The numbers are based on patients with data available.

COPD, chronic obstructive pulmonary disease; AAT1, Alpha 1-antitrypsin deficiency; PH, pulmonary hypertension; CF, cystic fibrosis; PF, pulmonary fibrosis; CMV, cytomegalovirus; EBV, Epstein-barr virus; BMI, body-mass index; FVC, forced volume vital capacity; FEV1, forced volume expiratory capacity 1 sec; 6MWT, 6-min walking test; AST, aspartate transaminase; ALT, alanine transaminase; SLTx, single-lung transplantation; DLTx, double-lung transplantation; HLTx, heart-lung transplantation; Re-LTx, re-lungtransplantation; ATG, anti-thymocyte globulin; ECMO, extracorporeal membrane oxygenation

Survival rates

Considering post-operative survival for ABO-identical versus ABO-compatible LTx for the entire cohort (excluding HLTx), ABO-identical LTx had 1-, 5-, 10-, 15-, and 20-year survival estimates of 91%, 64%, 44%, 30% and 16%, correspondingly, comparing with recipients that underwent an ABO-compatible LTx at 1-, 5-, 10-, 15-and 20-year survival estimates of 73%, 53%, 40%, 36% and 30% correspondingly (p > 0. 05) (Figure 11).

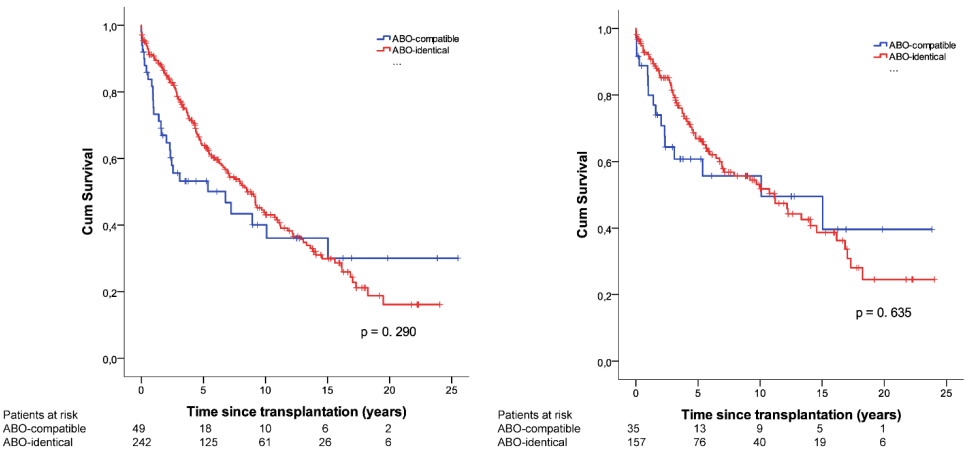

Figure 11: Cumulative retransplantation-free survival for ABO-compatible (N = 49) and ABO-identical (N = 242) transplants between 1990-2016 for the entire cohort excluding heart-lung transplantations (left figure) in addition to ABO-compatible (N = 35) and ABO-identical (N = 157) LTx when excluding patients that underwent single-lung transplantation (right figure).

Table 9: Cause of death after ABO-compatible and ABO-identical transplants in addition to donor blood group

	ABO-compatible n = 28 (%)	ABO-identical n = 123 (%)	p-value	A n = 51 (%)	B n = 17 (%)	AB n = 1 (%)	O n = 82 (%)	p-value
Cause of death			0. 795					0. 902
Rejection	6 (21)	34 (27)		12 (24)	4 (24)	1 (100)	23 (28)	
Infection	9 (32)	29 (24)		13 (25)	5 (29)	0 (0)	20 (24)	
Malignancy	4 (15)	18 (15)		9 (18)	3 (18)	0 (0)	10 (13)	
Miscellaneous	9 (32)	42 (34)		17 (33)	5 (29)	0 (0)	29 (35)	
The group called 'miscellaneous' is defined as patients with mortality caused by myocardial and cerebral ischaemia, and multiple organ failure such as renal and liver in addition to other causes related to the patient's old age and individual health status.								

Recipients that undertook LTx in the time-periods 1990 to 2005 and 2006 to 2016 are illustrated in Figure 12. No significant difference in outcome was shown for ABO-identical and compatible LTx in neither period ($p > 0.05$).

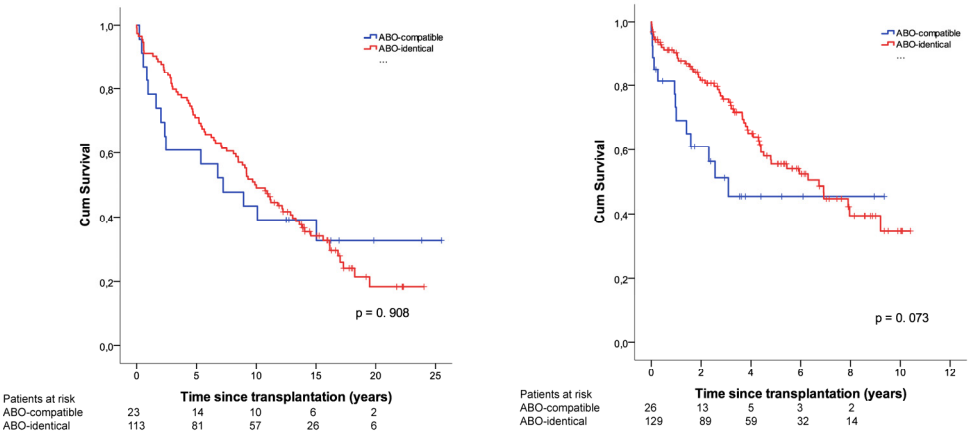

Figure 12: Cumulative retransplantation-free survival for ABO-compatible (N = 23) and ABO-identical (N = 113) transplants for the period 2006-2016 and ABO-compatible (N = 26) and ABO-identical (N = 129) transplants for the period 1990-2005. No significant difference in survival was observed between the two groups in either of the time periods.

Post-operative survival estimates for patients that where ABO-identically matched and survived up to one year were 95% at 100 days, 93% at 200 days and 91% at 300 days (CI 88–95) compared with recipients with ABO-compatible LTx with 100-, 200-, and 300-day survival rates of 88% (CI 79–97), 86% (CI 76–96), and 84% (CI 73–94), respectively ($p < 0. 05$) (Figure 13). Concerning recipients with a limited post-operative survival of 10-years, no difference was shown as well as emphysema-patients, excluding SLTx and recipients that underwent LTx before 2005 and after 2005 ($p > 0.05$).

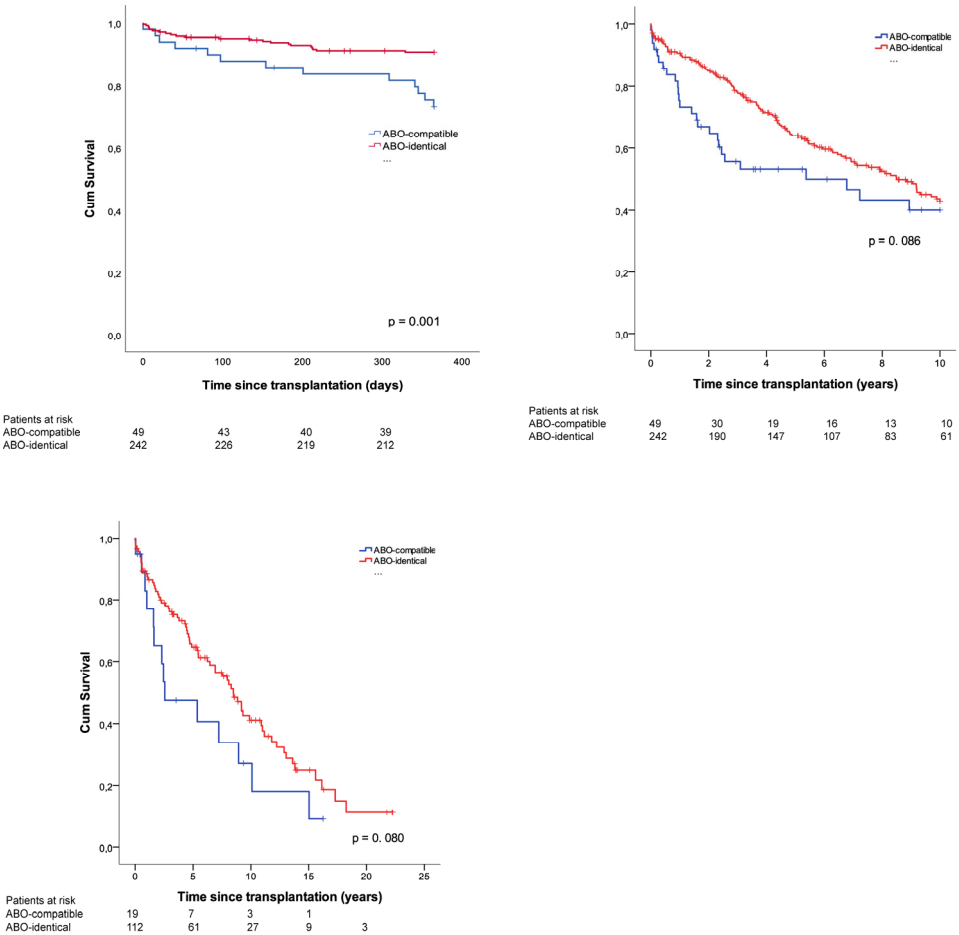

Figure 13: Cumulative retransplantation-free survival for ABO-compatible (N = 49) and ABO-identical (N = 242) LTx for patients with a limited survival up to 1-year (upper left figure), ABO-compatible (N = 49) and ABO-identical LTx (N = 242) up to ten years (upper right figure) and overall survival for ABO-compatible (N = 19) and ABO-identical (N = 112) LTx in emphysema-patients between 1990-2016 (bottom left figure). A significant difference in survival was observed between compatible versus identical matching in patients with limited survival up to 1-year.

Hazard ratios

The Cox proportional hazards model (univariable) assessing ABO-identical versus ABO-compatible LTx as well as additional risk factors for outcome in emphysema recipients in specific are illustrated in Table 10. Age had a HR of 1.044 (1.010 – 1.078) and patients ≥ 55 years with a HR of 2.115 (1.306 – 3.425) ($p < 0.05$). Regarding infection, CMV-mismatching showed a HR of 2.588 (1.438 – 4- 659) and EBV-mismatching a HR of 3.556 (1.511 – 8.371) ($p < 0.05$).

Table 10: Cox regression analysis for identical versus compatible ABO-matching, recipient/donor-blood group and other risk factors for retransplantion-free survival among emphysema-patients (univariable)

	HR	95 % CI	p-value
Identical ABO-match	0. 600	0. 336 – 1. 069	0. 083
Age	1. 044	1. 010 – 1. 078	0. 010
Identical ABO-match x age	0. 995	0. 984 – 1. 005	0. 328
Recipient > 55 years	2. 115	1. 306 – 3. 425	0. 002
BMI	1. 035	0. 979 – 1. 094	0. 226
Male	0. 992	0. 632 – 1. 558	0. 973
Gender mismatch	0. 935	0. 600 – 1. 459	0. 935
Waiting list	0. 998	0. 997 – 1. 000	0. 009
Infection			
Recipient CMV	0. 659	0. 380 – 1. 144	0. 138
CMV-mismatch	2. 588	1. 438 – 4- 659	0. 002
Recipient EBV	1. 010	0. 634 – 1. 609	0. 965
EBV-mismatch	3. 556	1. 511 – 8. 371	0. 004
Recipient toxoplasma	1. 254	0. 784 – 2. 007	0. 345
Toxoplasma-mismatch	1. 426	0. 649 – 3. 133	0. 377

CMV, cytomegalovirus; EBV, Epstein-barr virus; BMI, body-mass index CI, confidence interval; HR, hazard ratio

Experimental results

DCD-A versus DCD

Regarding the highlighted results of the experimental section of this thesis, animal weights in the 2 groups were: 63 ± 1 kg in DCD-A and 61 ± 1 kg in the DCD group ($p < 0.05$). PaO2 at an FiO2 of 0.5 were amongst DCD-A 29 ± 0.5 kPa whilst in the DCD group 29 ± 1 kPa ($p > 0.05$). PaCO2 (FiO2 of 0.5) were amongst DCD-A 6.0 ± 0.5 kPa whilst in the DCD group 6.0 ± 0.2 kPa ($p > 0.05$). EVLP time was 56 ± 3 min for the DCD group and 55 ± 4 min for DCD-A ($p > 0.05$). No irregularities regarding anatomy, signs of infection, or malignancy were observed in any animal regarding autopsy.

Pulmonary gas function

The DCD-A group showed a PaO2 of 60.3 ± 3.7 kPa whilst the DCD group had a PaO2 of 51.7 ± 2.1 kPa after completing EVLP with an FiO2 of 1.0 ($p > 0.05$) (Table 11)

Pulmonary artery flow and pressure

Pulmonary artery flow (PAF) at FiO2 of 1.0 resulted in 4.00 ± 0.02 l/min in the DCD-A group while the DCD group had a PAF of 3.87 ± 0.10 l/min ($p < 0.05$) Figure 14).

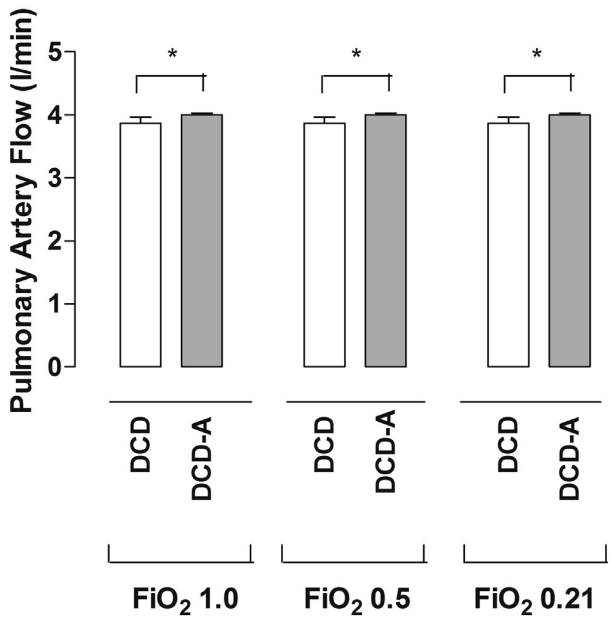

Figure 14: . The mean pulmonary artery flow (PAF) (±SEM) after Ex Vivo Lung Perfusion (EVLP) is illustrated for the three different groups: donation after circulatory death without alteplas (DCD) and with alteplase (DCD-A) at different fractions of inspiredoxygen (FiO2). Statistical analysis was performed using Mann-Whitney test. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p > 0.05$ (n.s.).

Table 11: The table demonstrates the following parameters of blood gases PaO2, PaCO2, PvO2 and PvCO2 for inspired oxygen fractions of 1.0, 0.5, and 0.21 for the three different groups: DCD-A, and DCD. Statistical analysis was performed using Mann-Whitney test to compare DCD-A, and DCD.

	DCD-A	DCD	p-value
PaO2 (kPa)			
FiO2 1.0	60.3 ± 3.67	51.7 ± 2.05	0.142
FiO2 0.5	26.4 ± 1.37	23.4 ± 0.80	0.493
FiO2 0.21	9.5 ± 0.43	9.0 ± 0.35	0.951
PaCO2 (kPa)			
FiO2 1.0	3.8 ± 0.32	3.5 ± 0.09	1.000
FiO2 0.5	3.2 ± 0.05	3.3 ± 0.09	1.000
FiO2 0.21	3.1 ± 0.12	3.6 ± 0.10	0.060
PvO2 (kPa)			
FiO2 1.0	6.8 ± 0.29	7.1 ± 0.14	0.966
FiO2 0.5	7.3 ± 0.13	6.9 ± 0.20	0.237
FiO2 0.21	4.2 ± 0.21	5.9 ± 0.40	0.001
PvCO2 (kPa)			
FiO2 1.0	3.9 ± 0.07	3.8 ± 0.09	1.000
FiO2 0.5	3.7 ± 0.05	3.6 ± 0.18	1.000
FiO2 0.21	3.6 ± 0.08	4.1 ± 0.08	0.001

FiO2 = Inspired oxygen fraction, PaO2 = arterial oxygen partial pressure, PaCO2 = arterial carbon dioxide partial pressure, PvO2 = venous oxygen partialpressure, PvCO2 = venous carbon dioxide partial pressure. DCD-A = donation after cardiac death with alteplase and non-heparin group, DCD = donation after cardiac death non-heparin group.

Pulmonary artery pressure (PAP) at FiO2 1.0 amongst DCD-A showed 14.83 ± 1.85 mmHg whilst the DCD group had a PAP of 17.83 ± 1.17 mmHg ($p > 0.05$) (Figure 15).

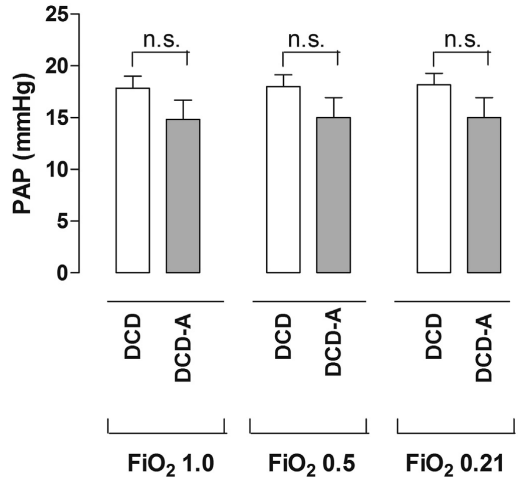

Figure 15: The mean pulmonary artery pressure (PAP) (±SEM) after Ex Vivo Lung Perfusion (EVLP) is illustrated for the different groups: donation after circulatory death without alteplas (DCD) and with alteplase (DCD-A) at different fractions of inspired oxygen (FiO2). Statistical analysis was performed using Mann-Whitney. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p > 0.05$ (n.s.)

Pulmonary vascular resistance

No significant differences were shown regarding pulmonary vascular resistance (PVR) in the DCD and DCD-A group with 372 ± 31 dyne x s/cm and 297 ± 37 dyne x s/cm5 groups respectively ($p > 0.05$) (Figure 16). PVR at FiO2 0.5 and 0.21 were comparable to PVR at FiO2 1.0.

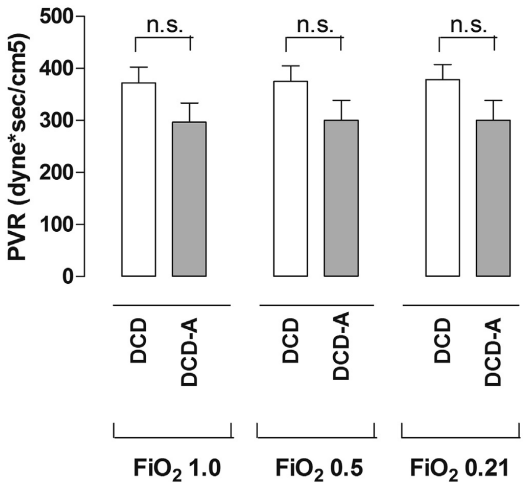

Figure 16: The mean pulmonary vascular resistance (PVR) (±SEM) after Ex Vivo Lung Perfusion (EVLP) is illustrated for the different groups: donation after circulatory death without alteplas (DCD) and with alteplase (DCD-A) at different fractions of inspired oxygen (FiO2). Statistical analysis was performed using Mann-Whitney test. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p > 0.05$ (n.s.).

Weight and macroscopic appearance

Before EVLP, the mean lung weight among DCD-A was 546.33 ± 29.06 g whilst the DCD group had a mean weight of 558.33 ± 21.39 g ($p > 0.05$). After EVLP lung weight for the DCD-A group showed 541.50 ± 31.25 g whilst the DCD group resulted in a mean lung weight of 589.17 ± 24.27 g ($p > 0.05$).

As to the pulmonary arterial branches that were macroscopically studied for thrombotic material, no thrombotic material was found in neither DCD or DCD-A.

Overall discussion

The golden standard

LTx is the golden standard for treating patients with end-stage pulmonary disease [57]. The quantity of clinical LTxs is hampered by the donor organ scarcity, resulting to the current situation of finding new means of making more organs available [49, 58, 59].

Lund University LTx programme

The quantity of LTxs at our center has expanded since its inception 1990. The quantity of SLTxs amounted to its highest number in 2002, subsequently diminishing due to the increase of DLTxs. This alteration in trend is credited to the growing data reflecting the superior long-term outcome rates in recipients that underwent DLTx. Recipients with CF were rare at our center programme with only two recipients between 1990 to 1993, comparing to 18 recipients between 2010 to 2014. The leading major indication for LTx at our center has been COPD, climaxing at approximately 30 percent in 2002 to 2009, thereafter diminishing to about 18% between 2010 and 2014. This discrepancy may be due to the quantity of patients with COPD on the waiting list.

Among the highest shown survival estimates

The median survival for the entire cohort was estimated to about 9.8 years. This data is in accordance with the highest survival estimates presented by other international centers, whilst follow-up is every so often limited to ten years [60-62].

DLTx - Are two lungs better than one?

In this thesis, superior long-term outcome was seen in DLTx patients compared to SLTx patients, this data support the clinical program of favoring DLTx recipients instead of SLTx. Superior long-term survival may be linked to superior recovery in pulmonary function in addition to minimized graft-related complications and mortality among DLTx versus SLTx [63, 64]. However concerning days in ventilator support, SLTx showed significantly shorter time, speculating that SLTx might have better chance of handling graft reperfusion injury, supported by the native lung.

CF – the star candidate

CF and AAT1 recipients illustrated the most superior survival estimates in comparison to COPD and PF with the least probability of survival. COPD patients often tend to represent an older recipient clientele presenting comorbidities as heart and vessel disease possibly explaining the discouraging results.

BOS – the great limitation

The outcome after LTx has become significantly better for the last ten years. Nevertheless, post-operative outcome is principally hampered by chronic rejection. CLAD, mainly established among recipients as BOS, is still the prime causing factor to morbidity as well as mortality. Granting the low probability of BOS occurring for the first 12 months, the cumulative incidence of BOS rapidly grows in the first five years [65, 66]. Predisposing factors for BOS are still under debate [67].

Anti-human leukocyte antigen donor specific antibodies have been shown to be associated with early BOS and mortality but it still under debate [68, 69]. Through plasmapheresis it's possible to discard of such antibodies, however the clinical effect of this treatment strategy on post-operative survival is still under discussion [70]. Other risk factors include bacterial/viral infections as causes of BOS [71, 72]. Initially BOS was thought to be non-reversible, whereas in certain patients, azithromycin has proven to recover pulmonary function with more than 10 percent. It has been publicized that BOS and PF correspondingly present comparable pathological features with similar pathophysiology, for example epithelial cell damage and growth and deposition of extracellular matrix [73]. Clinical findings of these LTx recipients could be of great benefit when it comes to finding biomarkers with newer methods of diagnosing BOS at an earlier stage [74].

Major indication and BOS

In this thesis the highest cumulative incidence of death was found in COPD patients trailed by PH, AAT1, PF, and CF (in descending order). Signifying that CF and PF patients have better clinical outcome in spite of being diagnosed with BOS. In addition LTx recipients differs compared internationally, such as higher incidence of COPD and CF recipients combined with younger median age among Swedish recipients. [75]. Comparing CF vs. AAT1 recipients, CF had a lower cumulative incidence of death despite having a higher probability of developing BOS. This finding yields a positive impact of supporting CF patients to undergo LTx. Previous reports have shown CF patients to be associated with difficulties such as arthropathy related to CF and complex and deadly chronical infections related to *Aspergillus, B.Cepacia* and *P. Auriginosa* [76].

Furthermore, CF and PH patients being diagnosed with BOS were shown to have equivalent survival regardless of BOS. PH is actually seen after LTx in various patients as obliterative bronchiolitis has been linked to arterial and venous injury that is immune-mediated, causing PH both pre- and post capillary [77]. It might be possible that patients diagnosed with PH before undergoing LTx are better at resisting this phenomenon which could explain why poorer outcome was not shown versus CF in spite of BOS. Understanding this disease state could yield great clinical impact. This particular hypothesis was not examined in this dissertation as further data was needed.

Pulmonary function trends

The two most important outcomes after LTx is survival followed by post-LTx pulmonary function [78]. As previously mentioned, novel recovery treatments has been introduced to recover lost pulmonary function after CLAD such as the introduction of Azithromycin as a therapeutic option [79]. Reports have also suggested that defining a pulmonary function pattern might aid the clinician in better understanding BOS and to discover its progress at an early phase [56].

Confounders

Essential predictive data can be provided by physiological monitoring, where an accelerated decline is often associated with poor outcome [80]. There are several factors to be taken into consideration when interpreting post-LTx lung function. Besides CLAD that affects the clinical outcome there is also the matching of age/size of the donor/recipient, decline related to the natural age of the recipient and recurring infections. Another factor include the native lungs of the recipients, affecting the lung function in SLTx.

FEV1 and 6MWT

An obstructive pulmonary function pattern that is found early has been associated to earlier diagnosis of BOS. Structural donor lung damage has been mentioned as a central risk factor [81]. Significant findings were found in this dissertation in regards to pulmonary function. Analyzing freedom from BOS grade ≤ 1, FEV1 analyses propose that for every liter the recipient conducts, the hazard ratio drops by about 45 percent. The equivalent pattern was acknowledged when analyzing 6MWT, where an increase in 10 percent in work percentage will drop the hazard ratio by about 21 percent. These promising findings may aid us in determining trends in pulmonary function that could help us understand the progress of BOS with a more customized follow-up. 6MWT has in addition been found in the literature as a beneficial tool in foreseeing the outcome among potential LTx recipients, having a significant impact on the outcome

post-LTx [82]. Further it could be simplified by the findings of this thesis that the greater the distance that the recipient can perform in 6 minutes, the lower the risk for death or Re-LTx. Greater lung function aids the recipient resulting in a greater patient prognosis, as deterioration is linked with disease progression and poor outcome [83]. Vital predictive data may in addition be extrapolated from the deterioration in FVC, stated that FEV1 as well as FVC are reliable prognosticators of the deteriorating condition of LTx recipients [80]. Of course, several other factors has been associated with the deterioration of lung function after LTx besides CLAD, such as recurring infections, malignancy in addition to the recipient's individual health status [84].

ABO-compatibility

In spite of the progress made throughout the decennia, LTx as a medical procedure is hampered by the lack of donor lungs. This unfortunate scarcity obliges us to discover more methods of maximizing and allocating obtainable donor grafts [85]. It has been long proposed that to ensure best survival outcome, it is optimal to identically match antigen-antibody concerning the recipient and donor. However such benefits in outcome is still under debate.

Identical versus non-identical compatible

In HTx, ABO-compatible matching has illustrated sub-optimal short-term outcome. Whereas LTx recipients, despite ABO-identical matching, has shown to have no inferior survival outcome than ABO-identical matching in the first year [86, 87]. As to whether this indifference is sustained in the long-term outcome remains under debate. This thesis presents that no difference is shown comparing ABO-compatible versus ABO-identical LTx matching in long-term survival. Important findings were discovered such as ABO-identical matching presenting a median waiting-list time being about 80% longer than ABO-compatibly matched LTx. A tremendous potential therein lies by decreasing the long-waiting list time and mortality of candidate waiting for a LTx. This in addition to the higher capability of increasing the volume of possible donors by accepting more ABO-compatible matching. In theory, A, B and AB-blood group recipients could have the great advantage of having a higher probability of being included. Nonetheless it is essential to not forget the O-recipients that would in such a scenario have the disadvantage by having a lower status of being prioritized. O-recipients may only receive donor lungs from an identical match versus the other blood group recipients [88].

Longest available follow-up

To the author's knowledge, this dissertation presents the longest patient follow-up from a single-center regarding long-term outcome from ABO-identical versus ABO-compatible LTxs. No clinical benefit was shown in overall or when excluding SLTx for identically matched recipients and donors. The same trend appeared stratifying the patients between different eras of being transplanted or major indication such as emphysema-patients. In addition, significant interactions was found for recipient age interacting with identical ABO-blood group matching. It seems that ABO-compatible matching remains as a capable possibility without significantly interfering with the survival despite the old age of a recipient. It has been described in the literature of HTx that age may affect survival outcome by interacting with ABO-compatibility, as young recipients have a tendency of presenting fewer incidents of infectious complications but higher occurrence of organ rejection [45].

Increasing DCD

With the current shortage in the available donor pool and mortality on the waiting list, a rising trend is seen in DCD [89]. More evidence have been emerging regarding the tolerance of warm ischemia in the donor lungs in addition to preserving pulmonary function capacity. Reports have been showing that up to one hour of warm ischemic time do not affect the donor lung graft [50, 90-92]. In addition to EVLP that is now an essential method in evaluating DCD lungs [4, 6, 47, 49, 93-95]. It has been suggestested that EVLP yields its protective influence on the donor lungs by restoring the graft to its original physiological/metabolic state by interrupting cold storage injury.This in addition to decreasing the microbial donor load and decreasing the probability for infection in the immunosuppressed recipient [5, 96]. As EVLP opens up the possibility for extending preservation which could allow for daytime surgery [97], its possible to reduce the geographical distance between recipients/donors [98]

Post-circulatory thrombosis

There is still progress being made in the different methods regarding the most advantageous approach to preserving the graft and avoiding thrombosis. Heparin that has been previously suggested lack any effect on microthrombi which have turned the attention on the possible use of fibrinolytic pharmaceuticals [51, 52, 99]. The usage of urokinase in a DCD-lung models among canines has shown superior results than its corresponding control group that underwent two hours of ischemia, with a similar model using recombinant tissue-type plasminogen activator with promising result in

gas exchange and even possibly the performance of the cardiopulmonary system [51, 99].

DCD-A versus DCD

In this dissertation, no difference was shown in blood gases between the two groups, with both groups fully meeting the inclusion critera for undergoing LTx comparing to international criteria [100]. Exellent results was also shown in parameters such as PAF and PVR when comparing both groups.

No need for alteplase

There has been previously reported that adding urokinas in the DCD model may show better outome than standard DCD [52]. However the reported model also underwent topical cooling after an extensive period of ischemic time, which may establish microthrombs and explain the superior effect of urokinase. The DCD model in this thesis remained unaffected by alteplase in one hour of warm ischemia, rendering the lung microvessels unharmed. The findings of this thesis differs with the previous literature, as the hemodynamics of the presented DCD models were not affected. This might suggest that adding topical cooling as well as elongating the period of topical cooling might harm the epithelium in the donor lung, even causing a greater hazard regarding thrombosis.

Limitations

Retrospective cohort analyses

25-year follow-up

Non-randomized retrospective cohort study has numerous inevitable limitations [101] . A limitation of the retrospective aspects of this disertation is the relatively extensive follow-up time, as donor selection has progressed over the last 25 years. Significant progress in the care of LTx recipient might impact parameters such as survival, which in turn depends on the period of LTx. Surgical and anaesthesia methods have also improved and advanced, in addition to managing and treating LTx recipients in the perioperative setting and in the intensive care setting. A limitation is also the introduction of novel pharmacological agents in addition to better prophylactic treatments. LTx recipient inclusion-criteria have widened throughout the years, with

preoperative ECMO or preoperative ventilator support no longer contraindications for LTx as the LTx recipient clientele has become more complex.

Numerous confounders have been associated to long-term outcome in LTx that could interfere with the interpretations of the described findings. Such factors are recipient/donor age, lung capacity as well as recipient kidney function, oxygen supplementation and ischemic time.

BOS grade ≥ 1

In general, in the studied patient cohort, recipients with BOS grade 1 did not, in most of the cases, attain a target treatment or alternation in the immunosuppressant regime although the recipient was diagnosed with BOS. It is probable to diagnose BOS with spirometry, with ISHLT stating that a decrease in FEV1 more than 20% from baseline is linked to BOS grade ≥ 1, which is followed up for a minimum of 3 weeks, with the lack of confounders [12]. Spirometry is the golden standard for pulmonary function follow-up after LTx. This method however does show minor disadvantages or causes of misconception that can affect the obtained results. BOS acts as a surrogate indicator of likely obliterative bronchiolitis. Although it has even been reported that bronchiolitis obliterans may only be shown in a minority of BOS recipients, as the presented complex histopathology varies [54].

Confounders

Conceivable confounders that could interact with lung function after LTx in addition to BOS are repeated infections or a deterioration in FEV1 associated with natural aging, bronchial stenosis, pleural effusion, and diaphragmatic dysfunction [102] . It can be recognised that the definition of FEV1 < 80% from the most optimal baseline for BOS is arguable, comparing to the CLAD criteria at present, in regard to BOS versus RAS. To distinguish recipients that indeed have rejection (BOS) or not, in this dissertation the BOS analyses included recipients with BOS grade 2 or more. This may be cautious to follow.

ABO-compatible LTx

Some applicable parameters were not acquired that could affect the findings for ABO-compatible LTx. Data of erythrocyte transfusions after LTx and the presence of haemolytic anaemia were not obtained. A great limitation was the fairly minor quantity of patients and the divergence in volume between ABO-compatible and ABO identical LTx cohorts. A greater patient cohort and equally big groups would yield more powerful analyses and limit the incidence of type II errors.

Conclusions

25-year experience of LTx

Sweden has a population of 10 million with two active LTx centers, with Lund University Hospital as one of these centers. This dissertation presents the 25-year experience of LTx in Lund, Sweden. Excellent survival outcomes have been shown with 1-, 5-, 10-, 20- and 25-year survival outcomes of 88, 65, 49, 37 and 19 percent, correspondingly, yielding our patient one of the best survival outcomes internationally. The most superior long-term outcome was found in patients that underwent LTx due to CF, AAT1 and PH. DLTx indicated better results than SLTx, particularly after ten-year survival after LTx.

DLTx provides a protective effect on mortality and BOS

No difference was shown between DLTx vs. SLTx regarding incidence of BOS grade ≥ 2. Nonetheless, DLTx patients had a greater survival outcome regardless of the same hazard of developing BOS, as compared to SLTx patients. This would suggest that patients undergoing DLTx better endure BOS than SLTx. These findings further support back up an LTx program preferring DLTx instead of SLTx. In terms of major indication, the greatest incidence of BOS grade ≥ 2 was shown among PF, CF, COPD, PH, and AAT1 patients in order, with PF patients having the highest hazard of developing BOS. In contrast, CF and PF recipients did suggest a better survival outcome in spite of developing BOS comparing with COPD, PH, and AAT1 patients.

Pulmonary function trends

Survival analyses revealed that the greater the value the patient manage to perform at spirometry (FEV1) or 6MWT, the better the chance of survival. As to freedom from BOS, the analyses propose that for each liter the recipient finishes (FEV1), lowers the hazard ratio by 45 percent. The same trend was acknowledged in 6MWT, where an increase of only 10% in a 6MWT will lower the hazard rate by 21 percent.

Understanding pulmonary function trends could aid us in comprehending how to improve the outcome after LTx.

ABO-compatible vs. ABO-identical LTx

This dissertation has reported the longest follow-up regarding survival for ABO-compatible versus ABO identical matched LTx, were none of the findings showed difference in long-term outcome. Furthermore, CMV- and EBV mismatch between recipients and donors for emphysema-patients affect the outcome negatively in particular.

No survival advantage for ABO-identical LTx in long-term survival was revealed. The identical trend trailed in further analyses, comparing different LTx eras, excluding SLTx, patients with an outcome limited up to 10 years, and even in emphysema group. Identical matching showed over 80 percent more waiting-list time than ABO-compatible matching. There is a potential in the utilization of ABO-compatible LTx to improve the distribution of donor grafts and lessen waiting time.

DCD using Alteplase

Finding an optimized DCD model would yield a greater amount of donor organs for LTx, where a simplified method of treating potential donor grafts is essential. An example of such simplification is harvesting grafts without utilizing heparin. An optimized method where donors might be left "untouched" for up to 1 h is a potential method in solving the donor organ scarcity. The supplemental utilization of plasminogen activator (alteplase) showed no superior outcome for DCD lung function and performance. Nonetheless, all grafts met the clinical guidelines for LTx with great margins, with/without alteplase. DCD has in this dissertation been shown to be used safely without heparin and in scenarios where there is no need for topical cooling.

Future Perspectives

LTx can be argued to have the most challenging road ahead with advancing forward in the clinic among solid organ Tx. However there is a growing volume of LTxs performed with improving outcomes in spite of an increasing cohort of sicker recipients that undergo LTx.

Pre-transplant survival

Significant developments are still to be made in improving the survival for recipients waiting to undergo LTx. With the field of PH as a model, several treatment strategies are becoming established extending life expectancies even without the need for LTx, as there is a decreasing trend for idiopathic PH as indication for LTx. A similar phenomenon might be expected from other major indications such as CF and PF [103-105]. As to patients deemed to have missed the "window of opportunity" for LTx, a growing trend can be expected for a more generous utilization of ECMO as these candidates begin to show promising results after LTx [106-109].

Donor availability

Donor criteria have significantly advanced throughout the years, leading to grafts that were deemed unacceptable now being commonly used. Further advances that have appeared will remain to fuel the evolution of acceptable lungs. A cornerstone for these future endeavours will undoubtedly involve EVLP [4, 110-112]. A vast potential of benefits is to be made in the personalized and pharmacological customization between the donor lung and the recipient such as gene therapy to enhance graft repair.

Yet to date, xenotransplantation is still a potential field in addressing the clinical difficulties between donors/recipients. Since the failed attempts of liver tx between primate and human in the beginning of the 90s, the field has advanced tremendously regarding the understanding of the challenges that has to be faced in order to become a viable clinical option. It is likely that the first clinical trials will comprise of genetically altered porcine lungs with efforts of inducing tolerance and strategically immunosuppress not only B and T cells but in addition to harnessing the control of

NK-cells, macrophages in addition to the regulatory mechanisms of the complement and coagulation system [113].

Tailored therapy

A great number of prospective and randomized trials are to be expected in LTx, evaluating novel immunosuppressive agents and treatment strategies. It seems likely that these advancements will begin to arrive among kidney/liver or HTx in the field of solid organ Tx before being applied to LTx.

The wide spectrum of outcomes is set by the relation of donor/recipient characteristics, comorbidities and additional external parameters. LTx patients are characteristically followed-up by extensive restrictions depending on the acting protocol of the centre, despite alternatives in treatment interventions and preventive options. Tailored therapy is consistently applied and adjusted based on ensuing complications and side-effects of immunosuppressive and anti-infectious pharmaceuticals. The idea of tailored therapy that is built-in to the acting protocol could be designed with the aid of risk analysis based on biomarkers. Developments through proteomics will uncover methods to minimal invasively detect acute rejections and differentiate between types of acute but foremost chronic allograft dysfunction. A better understanding in the background of CLAD will permit preventive approaches and targeted interventions for the different phenotypes of CLAD. Micro CT imaging can come to provide more detailed radiological stratification of CLAD [23]. Serum, BAL and exhaled air can provide analyses through proteomic and genomic evaluation showing great promise in treating CLAD [114-118].

Bioartificial lungs

A futuristic approach that might be closer to the clinic than we think is the use of tissue engineering and artificially made lungs. A dominating principle consists of seeding a decellularized lung matrix with stem cells or/and other appropriate cell lines. This approach would practically solve the great challenges we face today after LTx such as organ rejection and immunosuppression-related complications [119-122].

The lung is a highly multifaceted and complex organ consisting of numerous different types of cells, each with a different and precise function [123, 124]. Present methods being undertaken in the pre-clinical and clinical settings exploit biologically procured or artificial scaffolds that become seeded with autologous cells from the supposed tx candidate [125, 126] . Both synthetic and biologically procured scaffolds each have their own benefits and drawbacks. In addition, hybrid scaffolds (merging biologically

procured and synthetic scaffolds) may have a role to play in a new approach to limit the difficulties shown with synthetic or biological scaffolds by themselves. Numerous technologies have been brought forward to assist in producing scaffolds for lungs, such as decellularizing for biological scaffolds in addition to cutting-edge processes for constructing synthetic scaffolds including casting, electrospinning, and microfabrication methods [127, 128] .

Though substantial advancement has been made into bioengineering lung/airway tissue (ex vivo) with the aim of producing functional lung tissue and finally be able to undergo LTx, more than 40 different types of cells with possibly hundreds to thousands of various cellular subtypes are going to have to be mastered and understood [129, 130].

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Lung transplantation: improving clinical outcome and donor organ scarcity

Mohammed Fakhro, MD, was born in 1993. He grew up in Kristianstad, Skåne, attended the Natural Sciences program at Österänggymnasiet and later went to medical school at Lund University. He graduated in 2018 and is currently doing his internship at the Central hospital in Kristianstad in order to attain his medical license.

Mohammed began his PhD studies in parallel with his first year at medical school and achieved his PhD shortly after his MD. This thesis is a proof of concept of how to understand and improve the clinical outcome after lung transplantation. The biggest challenge in lung transplantation to date is the scarcity of donor organs. This thesis proves insight in how to improve and better utilize donor organs in order to save as many lives as possible for patients in the need of a lung transplantation.

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