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Donor Factors and Recipient Selection in Lung Transplantation

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Table of contents

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Abbreviations

6MWT, Six-minute walk test

A1ATD, Alpha 1-antitrypsin deficiency

ATG, Anti thymocyte globulin

AU, Arbitrary units

BAL, Bronchioalveolar lavage

BDD, Brain dead donors

bFGF, Basic fibroblast growth factor

BLTx, Bilateral lung transplantation

BMI, Body mass index

BO, Bronchiolitis obliterans

BOS, Bronchiolitis obliterans syndrome

CA, California

CF, Cystic fibrosis

CI, Confidence interval

CLAD, Chronic lung allograft dysfunction

CMV, Cytomegalovirus

COPD, Chronic obstructive pulmonary disease

CPB, Cardiopulmonary bypass

CT, Computed tomography

CTD, Connective tissue disease

DAG, Directed acyclic graph

DAMP, Danger associated molecular patterns

DCD, Donation after circulatory death

DLCO, Diffusion capacity of the lung for carbon monoxide

ECMO, Extracorporeal membrane oxygenation

EDTA, Ethylenediaminetetraacetic acid

ELISA, Enzyme-linked immunosorbent assay

EVLP, Ex-vivo lung perfusion

FDA, Food and Drug Administration

FEV_1, Forced expiratory volume in one second

FiO_2, Fraction of inspired oxygen

FVC, Functional vital capacity

G-CSF, Granulocyte-colony stimulating factor

GM-CSF, Granulocyte macrophage colony stimulating factor

HELTx, High emergency lung transplantation

HLA, Human leukocyte antigen

HR, Hazard ratio

ICP, Intracranial pressure

ICU, Intensive care unit

IIP, Idiopathic interstitial pneumonia

IL, Interleukin

IL-1ra, Interleukin 1 receptor antagonist

ILD, Interstitial lung disease

IFN, Interferon

IP-10, Interferon-inducible protein 10

IPAH, Idiopathic pulmonary arterial hypertension

ISHLT, International Society of Heart and Lung Transplantation

IQR, Interquartile range

LAM, Lymphangioleiomyomatosis

LAS, Lung allocation score

LOS, Length of stay

LD, Living donors

LTx, Lung transplantation

MCP-1, Monocyte chemotactic protein 1

MIP, Macrophage inflammatory protein

mRNA, Messenger ribonucleic acid

MV, Mechanical ventilation

NYHA, New York Heart Association

PaO_2, Partial pressure of oxygen

PGD, Primary graft dysfunction

PH, Pulmonary hypertension

PDGF, Platelet derived growth factor

PRA, Panel reactive antibody

pTLC, Predicted total lung capacity

RAS, Restrictive allograft syndrome

RANTES, Regulated upon activation T cell expressed and secreted

RCT, Randomized controlled trial

SCR1, Soluble complement receptor 1

ScULAS, Scandiatransplant urgent lung allocation system

SD, Standard deviation

SIRS, Systemic inflammatory response syndrome

SLTx, Single lung transplantation

SULAS, Super urgent lung allocation system

ReTx, Retransplantation

TCC, Terminal complement complex

TGF, Transforming growth factor

TNF, Tumor necrosis factor

TLR, Toll-like receptor

UK, United Kingdom

US, United States

VEGF, Vascular endothelial growth factor

vaECMO, Veno-arterial extracorporeal membrane oxygenation

vvECMO, Veno-venous extracorporeal membrane oxygenation

Definitions

Bronchioalveolar lavage	A medical procedure where fluid is infused (via a bronchoscope) to a part of the lung and then collected for analysis
Brain dead donor	A donor who is declared dead on the basis of the criteria for irreversible destruction of the brain
Complement system	A part of the immune system consisting of several small circulating proteins
Cytokine	A small protein involved in cell signaling between immune or non-immune cells
Donation after cardiac death (DCD) donor	A donor who is declared dead on the basis of sustained cardiac arrest
Ex-vivo lung perfusion (EVLP)	Perfusion of donor lungs after procurement of the lungs using an ex-vivo perfusion system
Extracorporeal membrane oxygenation (ECMO)	A medical device used for extracorporeal oxygenation and removal of carbon dioxide from the blood
Forced expiratory volume in one second (FEV_1)	The volume of air that can forcibly be blown out in one second after full inspiration
Forced vital capacity (FVC)	The volume of air that can forcibly be blown out after full inspiration
Danger associated molecular patterns (DAMP)	Host biomolecules that can start and maintain a non-infectious inflammatory response
Diffusion capacity of the lung for carbon monoxide (DLCO)	A measure of the ability of the lungs to transfer gas from inhaled air to the blood cells
Half-life	The time required for a quantity (*e.g.* level of a cytokine) to reduce to half of its value
Immunogenicity	The ability of a substance to initiate an immune response
Inflammation	A biological response to a harmful stimuli such as a pathogen or tissue damage
Inflammatory biomarker	A cytokine, growth factor or complement complex associated with the immune system
Living donor	A donor who donates an organ or a part of an organ not required to sustain life in the donor

New York Heart Association (NYHA)-class	Classification system originally used to describe the degree of heart failure
Organ allocation	The allocation of organs to patients on the waiting list
Panel reactive antibody (PRA)	An antibody with affinity to any of several known specific antigens in a test panel
Primary Graft Dysfunction (PGD)	Complication during the first 72 hours after lung transplantation with reduced oxygenation and diffuse infiltrates on chest x-ray
Six-minute walk-test	A test that measures how far a person is able to walk within 6 minutes
Systemic inflammation	An inflammatory state that affects the whole body
Syngeneic	Organisms with identical genotypes
Traditional donor	A donor who is declared dead on the basis of the criteria for irreversible destruction of the brain

1 List of papers

Paper I

Auråen H, Mollnes TE, Bjortuft O, Bakkan PA, Geiran O, Kongerud J, Fiane A, Holm AM
Multiorgan procurement increases systemic inflammation in brain dead donors
Clinical Transplantation. 2013;27(4):613-8.

Paper II

Auråen H, Durheim MT, Dellgren G, Hämmäinen P, Larsson H, Geiran O, Lawaetz Schultz HH, Leuckfeld I, Iversen M, Fiane AE, Holm AM
Effect of donor age on outcome of lung transplantation stratified by recipient diagnosis: a Nordic multi-center study
Transplantation (In press)

Paper III

Auråen H, Lawaetz Schultz HH, Hämmäinen P, Riise G, Larsson H, Hansson L, Dellgren G, Perch M, Geiran O, Fiane AE, Iversen M, Holm AM
The Urgent Lung Allocation System in the Scandiatransplant Countries
Journal of Heart and Lung Transplantation (In press)

2 Summary

Organ transplantation may be the only treatment option available for patients with end-stage organ disease. However, the success of this therapy is limited by the scarcity of available donor organs and due to post-transplant complications. The overarching goal of this thesis is to save patients with end-stage organ disease by contributing insights on how to: 1) increase the number of donor organs that can be used for transplantation, 2) ensure fair and efficient allocation of donor organs, and 3) improve recipient outcome after transplantation. Of course, that is not to say that this thesis will revolutionize the field of transplantation by finding the definitive solution to the three challenges listed above, but hopefully some of the insights from this work will move the field one step further forwards. This thesis is composed of three articles that are connected because they ultimately aim towards the same high-level goal by addressing one or more of these challenges. The first study addresses organ transplantation in general, while studies two and three focus on the transplantation of lungs.

Study one relates to inflammation in organ donors, which is known to reduce the quality of donor organs. In turn, this may result in fewer organs suitable for transplantation and inferior recipient outcome. In this study, we characterize the inflammation before and during organ procurement by measuring a wide range of inflammatory biomarkers. We demonstrate that there is systemic inflammation in organ donors that increases during the procurement surgery. Taken together with findings from previous studies, our findings suggest that anti-inflammatory therapy may not only be beneficial while the donor is in the intensive care unit (ICU), but also during the procurement operation. Furthermore, our results give clues towards potential targets for anti-inflammatory therapy.

The second study relates to lung donor age and recipient outcome. The traditional ideal donor criteria recommend that the age of a lung donor should be below 55 years. However, due to the scarcity of available donor lungs, most transplant centers also use older donors. Our second study shows that patients with cystic fibrosis (CF) receiving organs from donors ≥55 years have inferior survival, while there were no differences in survival for patients with other diagnoses. Thus, our results suggest that improved allocation of donor lungs could increase overall recipient survival.

The third study relates to the Scandiatransplant Urgent Lung Allocation System (ScULAS), which is a collaboration between the Scandiatransplant countries to ensure the rapid allocation of donor lungs to patients who are critically ill and are most likely to die while on the regular waiting list. ScULAS was implemented in 2009, and gives each member center three "urgent calls" per year. By issuing an urgent call, the member center receives the first compatible donor lung which becomes available in the entire Scandiatransplant region. Unlike other systems designed to allocate organs to patients with critical conditions, the ScULAS has no predefined criteria defining which patients are urgent. Thus, the decision to use an urgent call is left entirely up to clinical judgement in each case. In our third study, we assess the ScULAS by describing the characteristics of the population receiving priority, assessing the time to lung transplantation (LTx) and urgent waiting list mortality, investigating recipient outcome and the utilization of the ScULAS by the member centers. We found that patients with suppurative lung disease (e.g. CF and non-CF bronchiectasis), younger patients, and patients on life support more commonly receive priority. Although the time to transplantation was short, waiting list mortality was higher among patients considered urgent than non-urgent. Furthermore, short-term post-transplant survival was inferior among those receiving urgent status. The insights provided by our third study might be valuable in order to refine the ScULAS in the future, and thus further improve the allocation of donor lungs and, potentially, recipient outcome.

3 Introduction

This introduction consists of five parts. The first part is a brief historical introduction to organ transplantation. This is included to put this work in a larger context. The second part focuses mainly on the traditional organ donor and how systemic inflammation in the donor has been shown to be detrimental for organ quality and recipient outcome. This is necessary to understand the rationale behind the first article, but some elements are also relevant for article two and three. The third and fourth parts are about how lung donor characteristics may influence recipient outcome and how donor lungs are allocated. These parts provide a background for article two and three. The last part gives a brief description of the lung recipient population, which is relevant for all articles.

3.1 Historical context and general introduction

Over the course of centuries, the transplantation of lungs and other types of tissues has evolved from being a supernatural event taking place in myths or fables to becoming a regular therapy for patients with end-stage organ disease. In Greek mythology, the sea goddesses Graeae, or Grey Sisters, had only one eye and one tooth that they shared and easily passed from one to the next when needed (1). In a Brothers Grimm tale, three army surgeons unintentionally transplant the hand of a thief, the heart of a pig and the eyes of a cat into themselves and thereby acquire the characteristics of the donors (2). During the 1900s, the gradual improvement of surgical techniques, especially the techniques for vascular surgery by Alexis Carrel (3), the development of methods for anesthesia (4), and a basic understanding of transplant immunology (5), were important aspects in the groundwork, paving the way for what is considered to be the first successful organ transplantation with favorable long-term results (6). This historical procedure was a renal transplantation performed in 1954 by Joseph Murray and his team at the Peter Bent Brigham Hospital in Boston (7). The patient received a kidney from his genetically identical twin brother and both lived healthy lives for several years following the transplantation (6). With the discovery of the positive effect of the combination of cortisone and azathioprine on graft survival (8), renal transplantation between individuals who were not genetically identical eventually showed successful long-term results in the 1960s (9). The first human LTx was performed in 1963 by James Hardy and his team at the Mississippi Medical Center. Notably, the patient was a 58-year-old male prisoner serving a life sentence for murder, and who had squamous cell carcinoma, emphysema and renal

failure. The donor was a patient with a myocardial infarction with circulatory shock and pulmonary edema in which resuscitation proved unsuccessful. Following the transplant, the recipient's renal failure gradually worsened, and he eventually died 18 days later (10).

In 1980s, the introduction of Cyclosporine substantially improved results in organ transplantation and gave optimism for LTx as a successful therapy (11). Moreover, improvements of the heart-lung machine was important for further progress (12,13). The first successful heart-lung transplant with long-term survival was carried out by Bruce Reitz and Norman Shumway at Stanford in 1981 in a patient with Eisenmenger syndrome (14). The patient was discharged after 80 days (14) and lived for 5 years after the transplant (15). In 1983, Cooper and his team at Toronto General Hospital performed the first successful single lung transplantation (SLTx) in a patient with idiopathic pulmonary fibrosis who survived for almost 7 years (15,16). Three years later, Cooper and his team also performed the first bilateral lung transplantation (BLTx) in a patient with emphysema who lived for 16 years (15,17). The discovery of Cyclosporine is viewed by many as the gateway to the modern era of transplantation (11), and the number of lung transplants and other solid organ transplantations has been steadily rising from this point (18) (Figure 3.1).

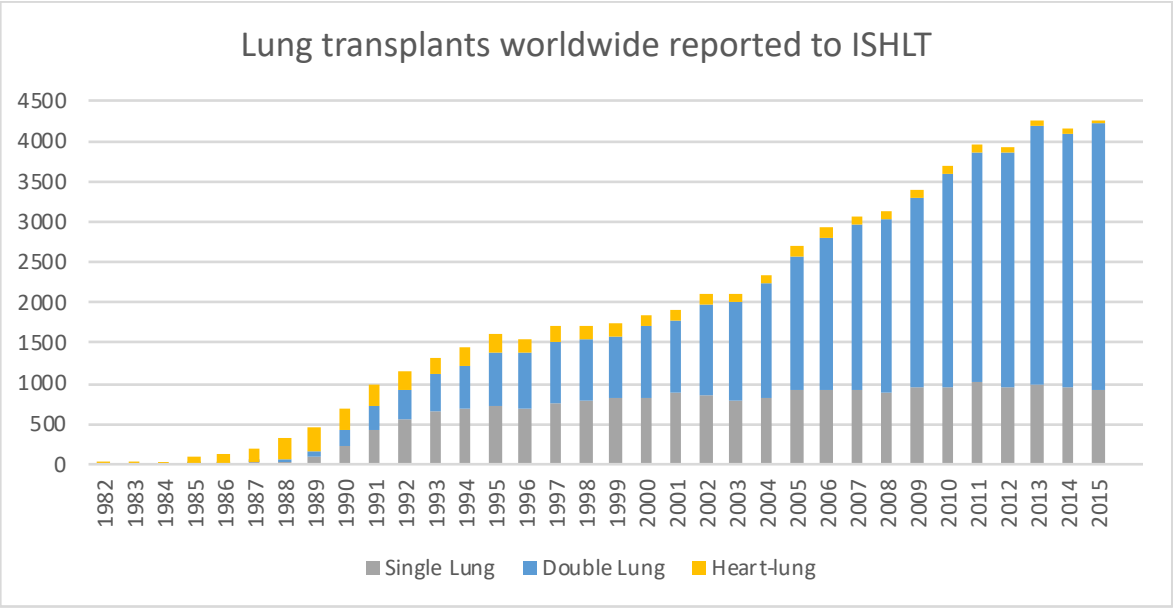

Figure 3.1. Number of lung transplantations worldwide reported to the International Society of Heart and Lung Transplantation (18) (ISHLT).

In Norway, the first LTx was performed in 1986 and was a combined heart and lung transplantation (19). The number of LTx has increased substantially during the last three decades (Personal communication: Are Holm) (Figure 3.2).

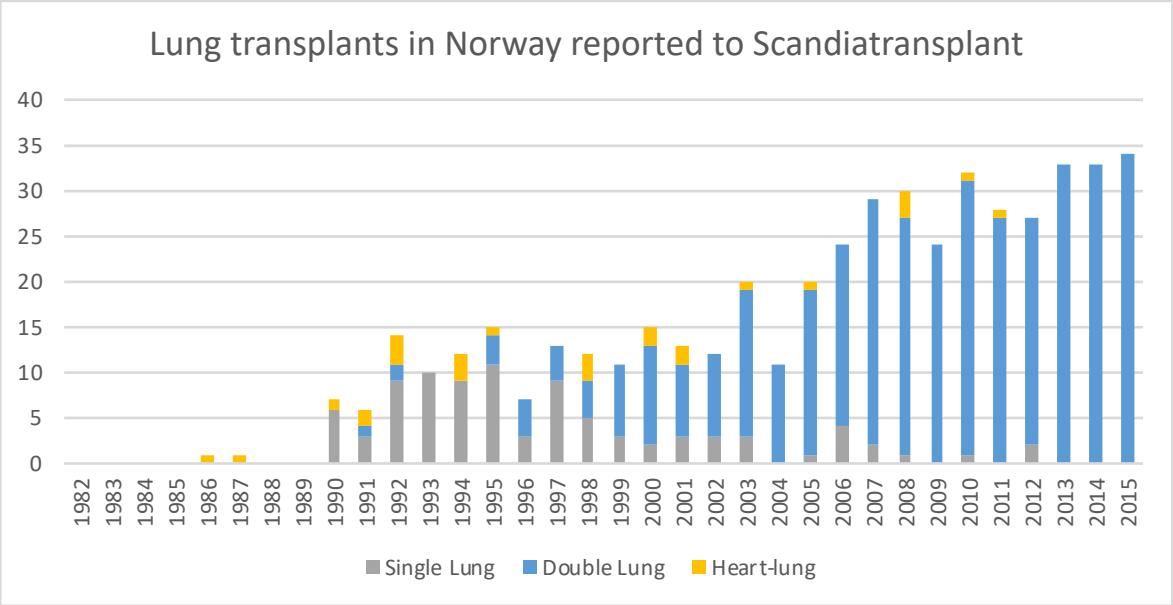

Figure 3.2. Number of lung transplantations in Norway reported to Scandiatransplant (Personal communication: Are Holm).

In the modern era of transplantation, organ shortage has been one of the major challenges to overcome and still remains a significant barrier for success (20). In LTx, there have been intense efforts to increase the donor pool. The lung donor acceptability criteria have gradually become less stringent. Initially, the recommended donor age was ≤45 years (21), but this was later extended to <55 years (22). Today, some centers use selected lung donors >70 years (23). Similarly, donors' smoking history should not exceed 20 pack years in the ideal donor criteria from 1993 (22), but, in the later years, many centers accept organs from heavy smoking donors (>20 pack years) (24,25). Moreover, the use of donation after circulatory death (DCD) donors has increased the donor pool (26). To evaluate and improve donor lungs before implantation, ex-vivo lung perfusion systems have been introduced into clinical practice during the last two decades (27,28). Furthermore, the proportion of BLTx has increased (18) (Figure 3.1 and 3.2) as this procedure yields superior survival for most recipient diagnoses (29-32). Besides this, the number of retransplantations (ReTx) after graft

failure has increased (18). In addition, extracorporeal membrane oxygenation (ECMO) is increasingly being used as a bridge to transplantation (33).

3.2 Organ Donors

Donors in organ transplantation include both dead and living donors (LD) (34). Dead donors may be declared dead on the basis of non-resuscitated cardiac arrest, and these are referred to as DCD donors (35). More commonly however, donors are declared dead on the basis of irreversible destruction of the brain with artificially maintained ventilation and circulation. These are referred to as traditional donors or brain dead donors (BDD) (36).

3.2.1 Traditional organ donors

In patients who become traditional donors there is usually an initial disease or injury (e.g. intracranial bleeding, traumatic brain injury) affecting the brain, which leads to hospital admission and life support treatment in the ICU. The brain insult leads to a gradual increase in intracranial pressure (ICP) that ultimately results in cessation of cerebral blood flow (37). At some point, the patient is declared dead on the basis of the criteria for irreversible destruction of the brain. According to Norwegian law, the following criteria have to be fulfilled in order to declare death in a patient with artificially sustained ventilation and circulation (38):

1. Known intracranial disease or injury
2. Complete loss of consciousness that is not due to medications or hypothermia
3. Cessation of spontaneous breathing
4. No cranial nerve reflexes
5. No blood flow to the brain determined by objective methods

If the patient's next of kin consent to donation and the donor is accepted by the transplant center, ventilation and circulation are artificially maintained in the ICU until the procurement operation (Figure 3.3).

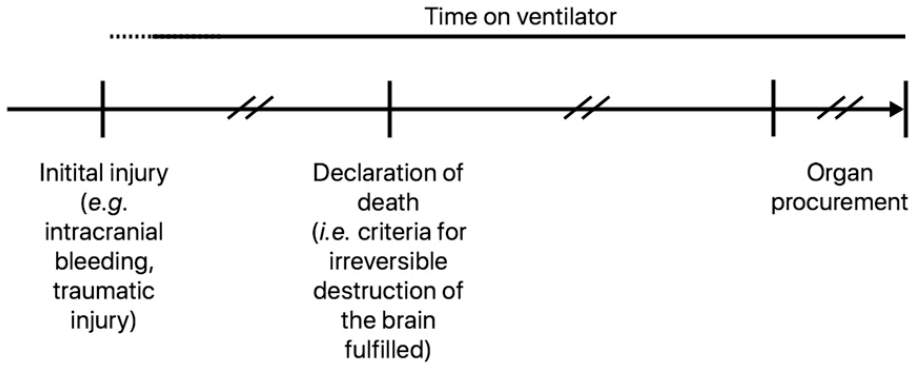

Figure 3.3. Simplified timeline for patients who become donors after irreversible destruction of the brain.

Importantly, the process leading to irreversible destruction of the brain and the period where ventilation and circulation are artificially maintained are associated with physiologic changes that may be unfavorable for potential donor organs used for transplantation (39). Due to the chaotic and uncontrollable nature of irreversible destruction of the brain in humans, several animal models have been used to provide valuable insights and clues to the physiology in traditional donors. In these models, brain death has been induced by balloon catheters that increase ICP or, alternatively, ligation of cerebral arteries (40-44). Together, observational human studies and experimental animal studies have identified important physiological changes which include (45):

1. Hemodynamic changes
2. Hormonal and metabolic changes
3. Systemic inflammation

3.2.2 Systemic inflammation in traditional organ donors

In general, inflammation may be defined as a biological response to harmful stimuli such as infection or tissue injury (46). In this thesis, systemic inflammation is used to describe an inflammatory state affecting multiple organs in the body. Systemic inflammation involves up-regulation and secretion of cytokines which act as cell-signaling molecules between both immune and non-immune cells (47). This signaling may be autocrine, paracrine or endocrine in nature, and the effect may depend on the recipient cell and the simultaneous presence of

other cytokines (48). Cytokines may be measured in tissue samples, serum, plasma, or other bodily fluids, and studies have suggested that levels of single cytokines or combinations of cytokines may characterize the degree and quality of inflammation (49,50). In addition, circulating soluble terminal complement complex (TCC) may be interpreted as a measure of complement system activation (51).

Previous studies have demonstrated that there is excessive systemic inflammation in traditional donor. The mechanism for this inflammation is not precisely understood, but hemodynamic instability, hormonal disturbances, anaerobic metabolism and the release of intracellular substances from the dying brain are likely to be involved. It is believed that this inflammation is harmful for the recipient as it reduces organ quality and increases organ immunogenicity (*i.e.* the degree to which the donor organ is recognized as foreign in the recipient) (45).

3.2.3 Effect of inflammation in traditional donors on recipient outcome

A large number of studies have provided evidence of the harmful effects of inflammation in traditional donors on recipient outcome for most transplantable organs, and some of these will briefly be reviewed in the following sections. Notably, a large part of the evidence is provided by animal studies where an experimental design is possible, but where generalizability to humans may be limited.

3.2.3.1 Lung transplantation

In LTx, several human and animal studies have explored the relationship between inflammation in the traditional donor and recipient outcome. Studies analyzing bronchioalveolar lavage (BAL) fluid in donors have demonstrated that the levels of inflammatory biomarkers, such as interleukin (IL)-6, IL-8, IL-12, Monocyte chemotactic protein (MCP)-1 and Vascular Endothelial Growth Factor (VEGF) are increased in donors where the recipient later develops primary graft dysfunction (PGD) (52), and that levels of IL-8 are associated with early mortality (53). Similarly, human studies analyzing messenger ribonucleic acid (mRNA) expression in lung tissue have found that high expression of genes encoding pro-inflammatory cytokines such as IL-6, IL-8, Tumor necrosis factor (TNF), and

IL-1β are associated with increased 30-day mortality, while anti-inflammatory cytokines are associated with favorable outcomes (54). One study using rat models found that brain death was associated with influx of inflammatory cells in the lung graft, and that recipient outcome was inferior with donor organs from brain dead rats compared to living rats (55). Consequently, several studies have studied ways to attenuate the donor inflammation before implementation. One retrospective study in humans found that the administration of methylprednisolone to lung donors resulted in improved oxygenation and increased organ yield (56). Another study, using an animal model, found that inhalation therapy with a low dose of carbon monoxide reduced inflammation in lung grafts and could improve outcomes after transplantation (57). Similarly, other animal studies found that anti-inflammatory IL-10 gene therapy was associated with improved outcomes after transplantation(58). A recent animal study explored the effect of BDD pre-treatment with a nebulized complement C3a receptor antagonist and found that it reduced the risk of ischemia reperfusion injuries and acute rejection in the recipient to the same levels as seen in LD (59).

3.2.3.2 Heart transplantation

In heart transplantation, some studies have investigated the relationship between donor inflammation and recipient outcome. One human study investigated the mRNA expression of TNF in biopsies from donor right ventricle and found that higher values were associated with failure of the right ventricle (60). Another study, using a rat model, found that donor brain death upregulated the expression of proinflammatory genes in the heart, and accelerated graft rejection in the allogenic recipients (61). However, a recent study with syngeneic recipient mice confirmed the upregulation of pro-inflammatory genes in heart tissue, but did not find evidence of accelerated ischemia reperfusion injury in the recipients (62). Another mice study found that the administration of anti-thymocyte globulin (ATG) to the donor after brain death reduced the proinflammatory gene expression in heart tissue, but did not explore the potential effects in the recipient (63).

3.2.3.3 Kidney transplantation

In kidney transplantation, where a large proportion of donors are LD, several studies have demonstrated superior survival when using organs from non-related LD compared to traditional donors (64,65). In humans, studies comparing kidneys from LD or DCD donors

and BDD have found increased leukocyte infiltration and up-regulation of genes associated with inflammation in BDD (66), and that grafts from BDD release more inflammatory mediators after implementation (67). Another study found up-regulation of adhesion molecules and human leukocyte antigen (HLA)-molecules in grafts from BDD compared to LD, and demonstrated that these changes were associated with graft rejection (68). However, one large human randomized controlled trial (RCT) did not find any effects of administrating corticosteroids to BDD on acute renal failure in the recipient (69). Interestingly, one animal study demonstrated that the use of soluble complement receptor 1 led to downregulation of pro-inflammatory genes in renal grafts and improved renal function post-transplant (70). Similarly, another study using a C1-inhibitor found that it reduced renal inflammation and improved renal function (71).

3.2.3.4 Liver transplantation

In liver transplantation, some studies have investigated the association between donor inflammation and recipient outcome. One human study compared LD to BDD and found increased expression of genes encoding pro-inflammatory cytokines including IL-6, IL-10, TNF, Transforming growth factor (TGF)-β and macrophage inflammatory protein (MIP)-1α and increased cellular infiltrates in the grafts from BDD. Furthermore, the authors demonstrate that this inflammation was associated with a worse ischemia reperfusion injury in the recipient (72). Notably, a human RCT assessing the effect of administering corticosteroids to liver donors found that this reduced systemic inflammation, the expression of adhesion molecules, and the risk of ischemia reperfusion injury as well as acute rejections after transplantation (73).

3.2.3.5 Pancreas and pancreatic islet transplantation

In pancreas transplantation, a limited number of studies have investigated the association between donor inflammation and recipient outcome. One study in humans comparing BDD to living patients undergoing pancreatectomy, found increased levels of TNF in pancreatic tissue in BDD (74), although mRNA levels of pro-inflammatory cytokines were similar between the two groups. In contrast, an experimental animal study found significant upregulation of mRNA encoding pro-inflammatory cytokines in pancreatic tissue, and found that pancreatic islets from BDD had reduced viability (75). Interestingly, 17β-estradiol, which may have anti-

inflammatory properties, has been shown to reduce inflammation and improve islet yield in animal models (76).

3.2.4 Inflammation during the organ procurement from traditional donors

The procurement operation from traditional donors often involves the retrieval of multiple organs and a simultaneous laparotomy and thoracotomy. As warm ischemia is harmful to donor organs, graft free-preparation is performed while the donor is circulated, and may last for several hours (77). Although several studies have assessed the inflammation in BDD before the procurement operation and found that this may be harmful for the recipient, few studies have assessed how the procurement operation itself affects the inflammation in the donor. Systemic inflammation, as determined by the presence of circulating pro-inflammatory cytokines, has been shown to occur in other types of major surgery (78). Thus, it is conceivable that the multi-procurement operation also leads to systemic inflammation. However, it is not known whether major surgery leads to an inflammatory response when there is severe pre-existing inflammation related to brain death. If such a response is seen, one may argue that future studies should investigate whether efforts to attenuate inflammation should not only be sought in the ICU after brain death, but also during the procurement operation.

3.3 Impact of donor characteristics on lung transplant recipient outcome

Several studies have investigated whether certain donor characteristics may impact upon recipient outcome. This may be important in order to select which donors that should be used in lung transplantation. Furthermore, if some donor characteristics only affect specific recipients, organ allocation may be optimized in order to increase overall survival. Donor age and smoking status have been of particular interest as the frequently cited donor criteria proposed by Cooper et al. in 1993 excluded all donors ≥55 years and those with a smoking history >20 pack years (22). Other donor factors that have been assessed in previous studies include size, gender, CMV-status and cause of death (67-70).

3.3.1 Donor Age

There are several changes that occur in the lungs as age increases. First, lung capacity starts to decrease after early adulthood. Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) peak at approximately 20 years of age, and then steadily decline. At 55 years, the lung function is approximately 75% of its peak in a non-smoking, healthy, Caucasian male (79). In addition, obstructivity (FEV_1/FVC) tends to increase with age (79), and diffusion capacity of the lung for carbon monoxide (DLCO) tends to decrease with age in adults (80). Second, older lungs have typically been exposed to cigarette smoke, pollution and other environmental substances for longer periods of time, which may negatively affect the lung architecture (81). Third, immunological changes including increased susceptibility for infections are observed in older individuals (82). Thus, in the original donor criteria, a donor age <55 years was recommended, as the authors suspected that using older lungs could affect recipient outcome (22).

Since the 1990s, numerous studies on the consequence of transplanting organs from older lung donors have been published, with conflicting results. Not surprisingly, these studies are retrospective and may be complicated by confounding and interaction effects that could be difficult to accommodate in statistical models. Some studies have found inferior lung recipient survival when transplanting older organs (83-85), but the implication of this is uncertain as the consequence of discarding such organs would mean that the recipient remains on the waiting list. On the other hand, several studies have found that donor age has no influence on recipient survival (23,86,87). This said, other studies have found that older donor organs may have negative consequences in some recipients, but not in others (88,89). If the last is the correct, it is possible that improved allocation of organs could lead to better overall survival. Some authors have argued that the recipient should have the possibility to opt out if the organ available is of inferior quality (90). If such informed choices are to be possible, knowledge about the relationship between donor characteristics and recipient outcome is necessary. To date, few studies have investigated whether the effect of using older donor organs varies between recipient diagnoses.

3.4 Allocation of donor lungs to recipients

Allocation of organs refers to the process of selecting which patient on the waiting list that should receive a donor organ. Because there are considerably more patients in need of LTx than available donor organs, prioritization is necessary to ensure fair allocation of lungs.

3.4.1 Ethical principles behind the prioritization of organs

The decision of which patients should be prioritized is often based on ethical principles such as utility and equity. Utility is the principle of maximizing benefit. That is to say, the transplantation that produces the most net benefit should be prioritized. An extreme form of utilitarianism would consider all forms of benefit, not only medical utility (e.g. survival or quality of life), but also social utility, which is the benefit that the patient could generate for others. Similarly, if it is likely that a patient will bring harm to others (*i.e.* negative benefit), an extreme utilitarian would not prioritize this patient. While most of us would agree that social utility should not be a factor in organ allocation, we would also agree that medical utility should be taken into account. One major disadvantage of having an allocation system based on medical utility alone is that it may discriminate against groups of patients known to have a less favorable outcome after transplantation. For example, such a system may discriminate against the elderly, the very young, a specific gender, certain diagnoses, socioeconomic groups, on race, or other factors. As opposed to utility, the principle of equity implies that all patients in need of transplantation (regardless of benefit) should have a comparable opportunity of attaining good health. For example, a patient who is incompatible (blood type and size are the two main matching factors for LTx) with a large proportion of the donor pool may need priority in order to achieve similar opportunity for treatment as a person who is highly compatible with the donor pool. Furthermore, a patient who is considered urgent (*i.e.* short expected survival without transplantation for example because of critical illness) may require priority in order to have the same opportunity for treatment as a patient who is not considered urgent. However, patients who are critically ill may have an inferior outcome after transplantation. Consequently, from a utilitarian point of view, one might argue that a system that only prioritizes the critically ill will be inefficient, as other patients on the waiting list will eventually also become critically ill with an inferior post-transplant outcome (90). Therefore, as both utility and equity are considered relevant by many, most organ allocation systems take both principles into account.

3.4.2 Lung allocation systems around the world

3.4.2.1 United States, Germany, the Netherlands and the Eurotransplant countries

The Lung Allocation Score (LAS) was first introduced in the US in 2005, and has later been adopted by Germany, the Netherlands and Eurotransplant (international exchange) (91,92). The LAS is a score from 0 to 100 based on the predicted survival of the patient on the waiting list the following year (i.e. urgency), and the predicted one-year survival after LTx (i.e. utility). Notably, predicted survival on waiting listed is weighted twice as much post-transplant survival. The following parameters are used to calculate LAS: FVC%, systolic pulmonary artery pressure, O_2 required at rest, age, body mass index (BMI), New York Heart Association (NYHA) class, diagnosis, six-minute walk test (6MWT) <150 feet, use of continuous mechanical ventilation (MV), diabetes, mean pulmonary capillary wedge pressure and serum creatinine (93). In the United States (US), the implementation of the LAS was associated with a shift towards a higher proportion of the transplanted having pulmonary fibrosis and also towards transplanting older patients. Furthermore, the implementation of the LAS was associated with a small, significant increase in post-Tx survival and a decrease in waiting list mortality (94).

3.4.2.2 France

In France, the High Emergency Lung Transplant (HELTx) system was introduced in 2007 and gives priority to patients who are considered critically urgent according to predefined diagnosis-specific criteria. In contrast to the LAS, which is a score from 0 to 100, patients in the HELTx system are either considered urgent or non-urgent. Moreover, the total amount of time a patient may have priority on the waiting list is limited to two periods of up to 7 days. Notably, the use of life support such as ECMO and MV are among the parameters that qualify for urgent status. Patients with Chronic Obstructive Pulmonary Disease (COPD) and LTx graft failure (i.e. ReTx) do not qualify for urgent status (although exceptions can be made in special cases) (95). One early study found that patients with urgent status had inferior survival (95), while a later study did not identify any survival difference between urgent and non-urgent groups (96).

3.4.2.3 United Kingdom

In the United Kingdom (UK), the super urgent lung allocation system (SULAS) was implemented in 2017. Similar to LAS and HELTx, SULAS gives priority to patients who are considered urgent based on predefined criteria. This system has two levels of urgency, namely super urgent and urgent. Super urgent status is given to patients who are already listed, either as urgent or non-urgent, and require life support (veno-venous extracorporeal membrane oxygenation (vvECMO) or interventional lung assist). Urgent status is given to patients for whom survival is likely to be less than 90 days, determined by diagnosis-specific criteria. As in the HELTx-system, patients previously transplanted are not considered eligible for urgent status. Furthermore, the SULAS guideline states that a patient without a reasonable chance of intermediate survival (50% probability of surviving 3-5 years post-transplant) should be removed from the list. For each level of urgency, the SULAS system prioritizes pediatric patients and small adults (≤155 cm) above the general adult population (97).

3.4.2.4 The Scandiatransplant countries

In the Scandiatransplant countries (originally, Denmark, Finland, Norway and Sweden, and also, from 2017, Estonia), the ScULAS was implemented in 2009. ScULAS gives priority to patients considered critically ill by clinical judgement (98). In contrast to other systems, there are no predefined criteria for urgent status. As with the SULAS, there are two levels of urgency, so that patients on life support (ECMO and/or MV) achieve priority on the urgent waiting list. The total number of patients who may be given urgent status is limited to three per year for each center (98). So far, no study has investigated which patients who are prioritized in the ScULAS. Furthermore, no study has assessed the utilitarian aspects of the ScULAS by investigating the outcome of patients given urgent status.

3.5 Lung transplant recipients

3.5.1 General indications and contraindications for lung transplantation

In general, an expert committee organized by the ISHLT recommends consideration of LTx if the following criteria are fulfilled:

1. High risk (>50%) of death from lung disease within 2 years if LTx is not performed
2. High likelihood (>80%) of surviving at least 90 days after transplantation

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

Furthermore, absolute contraindications include a recent history of malignancy (disease-free interval must be weighed against risk of recurrence), an untreatable dysfunction of another organ system (unless multi-organ transplantation is planned), uncorrected atherosclerotic disease with possible end-organ ischemia, acute medical instability, uncorrectable bleeding diathesis, certain infections, significant chest wall deformity, a BMI ≥35, a high risk of non-adherence to medical therapy after LTx, an absence of adequate social support system and severely limited functional status with poor potential for rehabilitation. In addition, there are several recommended relative contraindications which, among others, include age >65 years, poor nutritional status, and certain infections (99).

3.5.2 Distribution of lung pathology in the lung transplant population

The distribution of diagnoses in the adult transplanted population reported to ISHLT between 1995 and 2016 are shown in Figure 3.3. The most common diagnosis groups are (18):

1. COPD (36%)
2. Interstitial Lung Disease (ILD) (33%)
3. CF (16%)
4. Pulmonary hypertension (PH) (4.4%)
5. ReTx (4.0%)

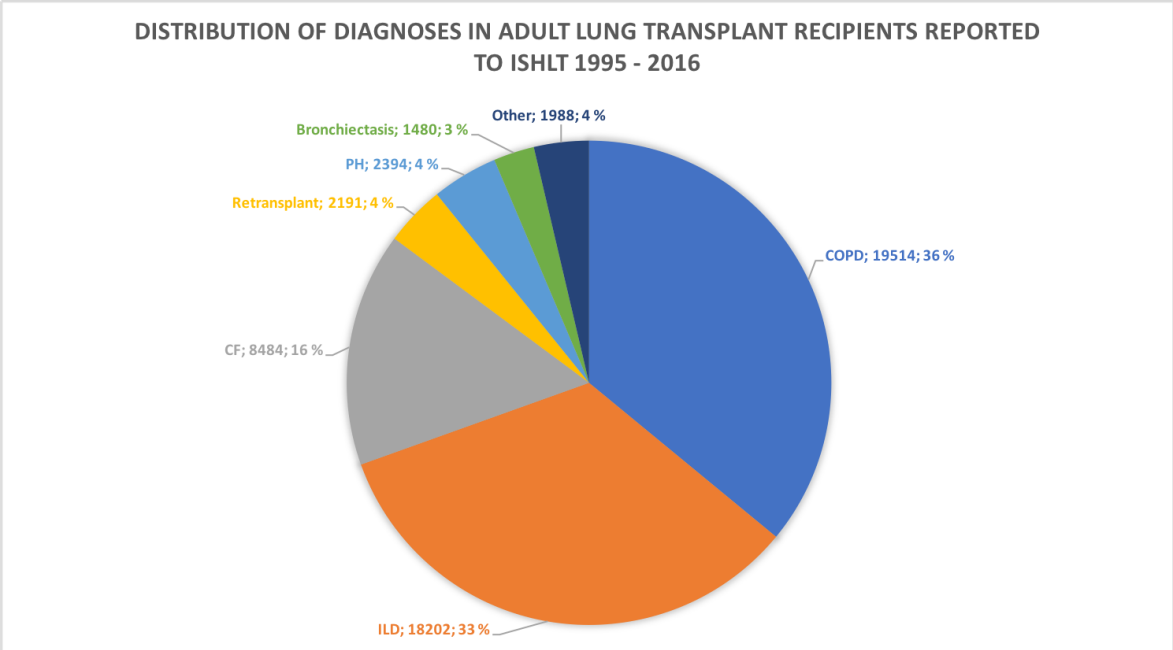

Figure 3.3. Diagnoses of adult lung transplant recipients reported to International Society of Heart and Lung Transplantation (ISHLT) (18) between 1995 and 2016 (n=54,253). CF, Cystic Fibrosis; COPD, Chronic Obstructive Pulmonary Disease; ILD, Interstitial Lung Disease; PH, Pulmonary Hypertension.

3.5.3 Life support as bridge to lung transplantation

Some patients are critically ill at waiting list entry or rapidly deteriorate on the waiting list. For some of these patients, MV or ECMO can be used as a bridge to transplantation while waiting for a suitable donor organ. Unfortunately, MV may sometimes provide insufficient tissue oxygenation in spite of maximal fraction of inspired oxygen (FiO_2), demanding the use of external oxygenation devices. vvECMO may be used if cardiac function is sufficient to maintain lung and tissue perfusion. However, PH and right ventricular failure may sometimes necessitate the use of veno-arterial ECMO (vaECMO) (100). Recently, several patients have been bridged to LTx using ECMO while being awake (101).

3.5.4 Treatment and follow-up after lung transplantation

In Norway, lung transplant recipients are observed in the thoracic ICU until they are sufficiently stable to be transferred to the ward. The time in ICU is highly dependent on early complications (as discussed in the next section), and may vary from one day to several

months in extreme cases. Chest tubes are commonly removed after 7-10 days. The majority of patients are discharged from hospital within 4 weeks. Immunosuppressive therapy is started before the transplant procedure and includes a calcineurin inhibitor (e.g. cyclosporine or tacrolimus), a nucleotide blocking agent (e.g. mycophenolate mofetil) and prednisolone. In addition, a methylprednisolone bolus dose is given intraoperatively. Furthermore, cytomegalovirus (CMV)-prophylaxis and antibiotics are given as required. Moreover, in Norway, lung function is assessed and surveillance biopsies are taken at 2, 4, and 12 weeks. Following this, recipients are controlled yearly with measurement of lung function and routine computed tomography (CT) scans (Personal communication: Are Holm).

3.5.5 Complications after lung transplantation

Complications seen in lung transplant recipients include:

- Primary Graft Dysfunction (PGD)
- Airway anastomosis problems
- Acute rejections
- Chronic Lung Allograft Dysfunction (CLAD)
- Renal failure
- Malignancy

It is estimated that between 15-25% of transplant recipients experience PGD, which is associated with increased short- and long-term mortality. PGD is characterized by reduced oxygenation (partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2)-ratio) and infiltrates on chest X-ray, which occurs within the first 72 hours after transplantation (102). Airway anastomosis problems include stenosis, dehiscence, fistulae and bronchomalacia, and can be seen in 7-18% of LTx recipients (103). Acute rejections occur in more than half of the LTx recipients, and are associated with inferior outcome even though it is usually effectively treated with steroids (104). CLAD remains one of the main barriers for success in LTx. Although the precise prevalence is unknown, it is believed that more than half of lung-transplanted patients are affected by CLAD 5 years after transplantation (105). The two most common phenotypes are bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS). In one single-center cohort, median survival for patients with RAS was 8 months compared to 35 months for patients with BOS (106). Chronic renal failure may occur in LTx recipients due to nephrotoxic effects of immunosuppression, and one study found that

15% of LTx recipients had chronic renal failure 5 years after transplantation (107). Finally, LTx recipients have an increased risk of developing several different types of cancers compared to the general population (108).

3.5.6 Survival after lung transplantation

For LTx recipients in the ISHLT registry between 1990 and 2015, the 5-year survival rates were 59% and 48% for BLTx and SLTx respectively. For patients undergoing their first ReTx, the 5-year survival rate was 40% (both SLTx and BLTx). Furthermore, females had slightly better survival than males. Notably, survival differed between recipient diagnoses. The 5-year survival for patients with CF was 63%, while the corresponding numbers for COPD (excl. Alpha 1-antitrypsin deficiency (A1ATD)) and Idiopathic Interstitial Pneumonia (IIP) were 54% and 50% respectively. LTx survival has gradually improved in the period from 1990 to 2015 (18).

4 Aims and objectives

The overarching aim of this thesis is to save patients with end-stage organ disease by contributing insights on how to:

A. Increase the number of donor organs that can be used for transplantation
B. Ensure fair and efficient allocation of donor organs
C. Improve recipient outcome after transplantation

Paper I

This study aims to provide insights on how to increase the number of organs that can be used for transplantation (A) and improve recipient outcome after transplantation (C) by elucidating inflammation in the organ donor and potential targets for intervention. Specifically, the main objectives of this study were to:

- Assess the systemic inflammation in BDD before procurement by measuring multiple circulating inflammatory biomarkers in plasma
- Assess if the multi-organ procurement operation modulates the secretion of circulating inflammatory biomarkers in plasma

Paper II

This study aims to provide insights on how to increase the number of organs that can be used for transplantation (A), ensure fair and efficient allocation of donor organs (B), and improve recipient outcome after transplantation (C) by assessing the consequences of using older donors in different lung recipient diagnosis groups. Specifically, the main objectives of this study were to:

- Evaluate the effect on recipient survival of using organs from older donors in BLTx in a Scandiatransplant cohort
- Assess whether the effects of donor age on recipient outcome depend on recipient diagnosis
- Assess the ICU LOS in patients receiving older donors

Paper III

This study aims to provide insights on how to ensure fair and efficient allocation of donor organs (B) by evaluating the results of the implementation of the ScULAS. Specifically, the main objectives of this study were to:

- Describe the population given urgent status in ScULAS
- Evaluate time to transplantation and waiting list mortality among patients with urgent status
- Assess outcome in recipients with urgent status
- Evaluate the utilization of the ScULAS by member centers
- Evaluate changes associated with the implementation of the Scandiatransplant Urgent Allocation System

5 Methods

5.1 Paper I

5.1.1 Study population, data collection and procurement

All BDD considered suitable for multiple organ donation (i.e. donations where thoracic and abdominal organs were used) in Norway were consecutively included from May 2010 to May 2011. Donor data were registered by transplant coordinators according to standard protocols. In total, organs were procured at 11 donor hospitals by surgeons from one retrieval team at Oslo University Hospital Rikshospitalet. Methylprednisolone (15 mg/kg) was administered to all donors prior to retrieval according to pre-existing donor treatment protocols.

5.1.2 Blood samples for cytokine measurements

Blood samples were drawn at the following time points during the procurement operation: before operation start (T0), 2 hours after operation start (T1), after free preparation of abdominal organs (T2) and after free preparation of the thoracic organs (T3) (Figure 5.1). Blood samples were drawn into sterile tubes containing ethylenediaminetetraacetic acid (EDTA), and stored on ice until centrifugation at 2465 x g at 4° C for 20 minutes. Plasma was isolated and stored at -70° C in sterile polypropylene tubes (1.8 mL Nunc cryotubes; Nalgene Nunc International) until final analysis in complete batches.

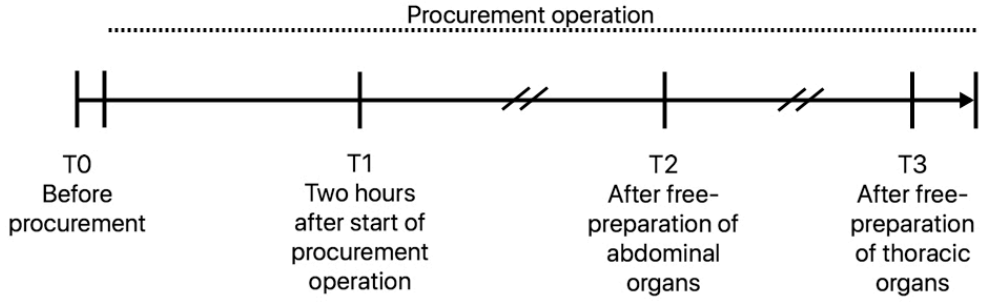

Figure 5.1. Timing of blood sample collection before and during the organ procurement operation.

5.1.3 Terminal complement complex (TCC)

The terminal SC5b-9 complement complex (TCC) was measured using an enzyme-linked immunosorbent assay (ELISA)-based method (109,110). Briefly, the monoclonal antibody aE11 was used as a capture antibody reacting with a C9 neo-epitope, which is exposed after C9 incorporation in the C5b-9 complex. A biotinylated monoclonal anti-C6-antibody (9C4) was used as a detection antibody. Human serum activated with zymosan was used as standard and defined to contain 1000 arbitrary units (AU)/mL.

5.1.4 Measurement of cytokines, chemokines and growth factors

Plasma samples were analyzed using a multiplex cytokine assay (Bio-Plex Human Cytokine 27-Plex Panel; Bio-Rad Laboratories Inc., Hercules, California (CA), US) with the following IL, chemokines and growth factors: IL-1β, IL-1 receptor antagonist (IL1-ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), interferon (IFN)-γ, interferon-inducible protein 10 (IP-10), Monocyte chemotactic protein (MCP)-1, MIP-1α, MIP-1β, platelet-derived growth factor-BB (PDGF-BB), regulated upon activation T cell expressed and secreted (RANTES), TNF, and VEGF. The samples were analyzed on a Multiplex Analyser (Bio-Rad Laboratories) according to instructions from the manufacturer. Selection criterion for markers to be included in the study was that more than 50% of the samples had levels above the lower detection limit of the assay. For single samples where measurements were below the lower detection limit, the values were set to equal to the lower detection limit for data handling and statistics. To determine levels in healthy, “non-inflammatory” individuals for reference, blood samples were collected from age- and sex-matched healthy individuals (n = 14).

5.1.5 Correction for dilution

Solid organ donors may be given large amounts of fluids in order to maintain adequate blood pressure during organ procurement, and this will markedly dilute secreted inflammatory biomarkers. To measure the total amount of secreted biomarkers as a reflection of the inflammatory activation elicited by surgical trauma to donor organs, cytokine measurements were corrected in proportion to changes in plasma total protein. Plasma total protein was

measured colorimetrically by the Biuret method on Cobas Modular (Roche, Basel, Switzerland) at the Department of Clinical Biochemistry, Oslo University Hospital, Oslo, Norway.

5.1.6 Statistical analyses

The levels of cytokines, growth factors, chemokines and TCC were, in general, not normally distributed. Consequently, non-parametric statistical methods were used for group comparison and correlation analyses. Specifically, independent non-parametric comparisons (Mann Whitney U-test) were used to compare donor cytokine levels to cytokine levels in healthy individuals. Paired non-parametric comparisons (Wilcoxon matched-pairs signed rank test) were used to compare donor cytokine levels at T0 and T3. Non-parametric correlations (Spearman) were used for all correlation analyses. A p-value below 0.05 was considered statistically significant. All statistical analyses were carried out using Graph Pad Prism 5.0 software (Graph Pad Software Inc., La Jolla, CA, US).

5.1.7 Ethical considerations

This study was approved by the Regional Committee for Medical Research Ethics.

5.2 Paper II

5.2.1 Study design

All patients undergoing LTx with organs from BDD in the Scandiatransplant area (i.e. Denmark (Copenhagen), Finland (Helsinki), Norway (Oslo) and Sweden (Gothenburg and Lund)) in the period 2000-2013 were included in the study. To minimize confounding of survival, ReTx multi-organ transplantations and SLTx were excluded. Recipients were stratified to the following diagnosis groups: CF, COPD, ILD or “Other”. Patients with lymphangioleiomyomatosis (LAM) were stratified to the “Other” group as these patients were considered clinically different from other types of ILD. The primary end-point of the study was recipient post-transplant survival. In addition, short-term complications were assessed by ICU LOS following transplantation.

5.2.2 Donor and recipient data

Anonymized donor and recipient data were collected from the Scandiatransplant registry. Donor parameters included age, gender, BMI, cause of death, last PaO_2/FiO_2, predicted total lung capacity (pTLC) calculated by donor height, sex and age (111) and donor smoking status. In the Scandiatransplant registry, donor smoking status is dichotomized to any history of regular smoking vs. no history of regular smoking. Recipient parameters included age, gender, BMI, percentage of predicted FEV_1 and 6MWT at last follow up, use of ECMO or MV as bridge to LTx, and Scandiatransplant urgent listing status.

5.2.3 Statistical methods

Normally distributed continuous data were presented as mean and standard deviation (SD), and compared using Student's t-test. Continuous data with other distributions were presented as median with interquartile range (IQR), and compared using non-parametric Mann-Whitney U-test. Categorical variables were presented as counts and percentages, and compared using Fisher's exact test. Recipient survival was assessed using Kaplan-Meier plots, the Log-Rank test and Cox regression. P-values less than 0.05 were considered statistically significant. STATA software version 15 for Mac (StataCorp LP, College Station, Texas (TX), US) was used for all statistical analyses. Prism version 6 for Mac (GraphPad Software Inc., La Jolla, CA, US) was used to create graphs.

Certain variables had missing data that appeared to be randomly distributed among the subjects. Primarily, this was evident for donor smoking status, where 37.7% of the data were unavailable. Subjects with missing data were excluded from multivariate models. Recipient survival was initially assessed using Kaplan-Meier plots and the Log-Rank test. To estimate the direct effect of using donors above the recommended age criteria (≥55 years), a directed acyclic graph (DAG) (112) was used to build a multivariate cox-regression model with relevant covariates. The relevant covariates were found to be recipient age, recipient urgency, cold ischemia time, donor smoking status and donor cause of death. Recipient life support (ECMO or MV) and/or Scandiatransplant urgent listing and recipient BMI was used as a surrogate for recipient urgency. A previous multi-center study has shown that cold ischemia time has a cubic relationship with recipient survival in BLTx, and that 330 minutes is a meaningful cutoff value (113). Cold ischemia time was therefore recoded as a dichotomous

variable using this cutoff value. As 37.7% of donor smoking data were unavailable, models were built with and without this variable. The proportional hazard assumption was assessed using scaled Schoenfeld residuals.

5.2.4 Ethical considerations

This study was approved by the Regional Committee for Medical Research Ethics.

5.3 Paper III

5.3.1 Study design

All patients listed for LTx in the Scandiatransplant area (i.e. Denmark (Copenhagen), Finland (Helsinki), Norway (Oslo) and Sweden (Gothenburg and Lund)), in the period 2005-2014 were included in the study. To evaluate the changes associated with the implementation of ScULAS, patients were divided into two groups: a pre-implementation period (1.1.2005-30.4.2009, 1,580 days) and a post-implementation period (1.5.2009-31.12.2014, 2,070 days). Patients were categorized to six diagnosis groups including: (1) Obstructive lung disease (e.g. COPD, A1ATD), (2) Restrictive lung disease (e.g. idiopathic interstitial pneumonia, connective tissue disease associated lung disease and sarcoidosis), (3) Suppurative lung disease (e.g. CF and non-CF bronchiectasis), (4) Vascular lung disease (i.e. PH), (5) Transplant Graft Failure and (6) Other diseases (e.g. LAM).

5.3.2 Data collection and statistical analyses

Anonymized patient and waiting list data were retrieved from the Scandiatransplant registry. Continuous data with non-normal distributions were presented as median and IQR, and compared using a non-parametric Mann-Whitney U-test. Categorical variables were presented as counts and percentages, compared using Fisher's exact test. P-values less than 0.05 were considered statistically significant. Graft survival was assessed using Fisher's exact test (specific time points) and the log-rank test (overall graft survival). Log-rank power calculations were performed using the Freedman method. STATA software version 15 for Mac (StataCorp LP, College Station, TX, US) was used for all statistical analyses. Prism version 6 for Mac (GraphPad Software Inc., La Jolla, CA, US) was used to create graphs and illustrations.

5.3.3 Organization of the Scandiatransplant Urgent Call Allocation System

Before ScULAS was introduced, each center allocated available organs to compatible patients on the waiting list within each country, prioritizing according to clinical judgment (Personal communication: Are Holm). There was no system for international organ exchange to patients with high urgency. ScULAS was set in effect on May 1, 2009 (98), after which all patients listed for LTx were categorized into three groups according to transplant urgency. Priority 0 included patients on life support (ECMO and/or MV). Priority 1 included patients with a rapid progression of organ failure and poor short-term prognosis as defined by the responsible center. In this thesis, Priority 0 and Priority 1 will hereafter be termed in aggregate as urgent. Priority 2 (hereafter called regular) included all other patients considered suitable for LTx. Patients initially listed as regular and then changed to urgent are presented as urgent in the results in this thesis. Each center had the right to claim supra-national priority for two urgent patients per year, increasing to three in March 2010. To list a patient as urgent, notice was given by the team of responsible physicians to the transplant coordinator on call, who submitted the request and all necessary recipient information electronically in the Scandiatransplant system. Notification of the new listing was then automatically given to transplant coordinators at all centers. If necessary, recipient serum was sent to potential donor centers. All compatible donor lungs were first mandatorily offered to Priority 0 patients and then Priority 1 patients in the entire Scandiatransplant area. If multiple recipients were listed with the same priority, the organ was allocated to the center with the highest rank on a rotating list. Local and then national urgent recipients were prioritized before urgent recipients in other Scandiatransplant countries. If no suitable urgent patient existed in the Scandiatransplant area, the organ was offered to regular patients locally or nationally, and then to regular patients in other Scandiatransplant countries according to the rotating list.

5.3.4 Ethical considerations

This study was approved by the Regional Committee for Medical Research Ethics.

6 Methodological considerations

6.1 Paper I

6.1.1 Quantification of systemic inflammation

To assess the multidimensional nature of the systemic inflammation in organ donors, we chose to measure a wide range of cytokines available in a multiplex cytokine assay kit. In addition, we measured TCC to assess the degree of activation of the complement system.

The Bio-Plex Cytokine 27-Plex Panel is based on 27 different microscopic beads (i.e. microspheres) that allow simultaneous quantification of multiple cytokines. Each bead type is coated with antibodies against a specific target (e.g. TNF, IL1 or IL8) and dyed with a specific ratio of two fluorophores. Thus, all bead types bind to only one type of cytokine and have a unique dye signature. After the beads are mixed with the plasma samples, biotin-labelled detection antibodies specific for secondary epitopes and fluorescently labelled streptavidin reporters specific for the detection antibodies are added to the solution. The multiplex analyzer, based on the principles of flow cytometry, generates a high-precision stream where the microspheres in the sample solution are lined up in a single file for individual analysis. A red laser is used for bead classification by exciting the bead type-specific dye. A green laser is used for cytokine quantification by exciting the reporter molecule associated with the detection antibody (Figure 6.1).

The main advantage with this system is the possibility to measure multiple analytes at the time with very little sample volume. Furthermore, it ensures that all analytes are measured under identical conditions. One potential disadvantage with multiplex bead assays is the "matrix effect", which is inaccurate quantification due to interactions between the measurements. For example, anti-cytokine cross-reactivity has been demonstrated to be a potential source of error. However, "matrix effects" are usually minimal in commercially available multiplex assays as used in this work (114). Furthermore, studies have shown that although ELISAs and different multiplex kits in general yield the same pattern of results, absolute values may not always be comparable (115,116).

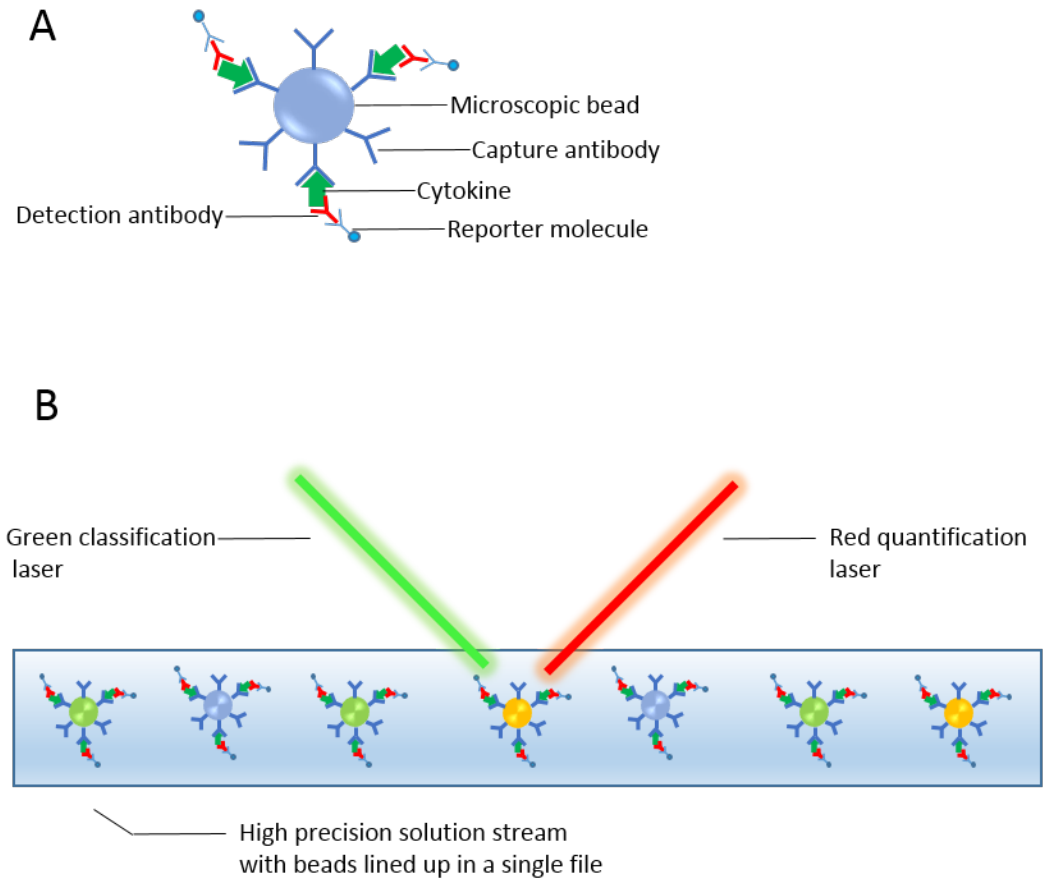

Figure 6.1. The main principles of the multiplex cytokine assay. A: Illustration of a microscopic bead. B: Multiple beads in solution with quantification (red) and classification (green) laser beams.

Complement activation was assessed by ELISA-based measurement of TCC, as this analyte was not available in a suitable multiplex assay. Complement activation may occur due to several triggers, and ultimately results in the formation of a terminal complex consisting of C5b, C6, C7, C8 and C9 (117). This complex may generate a membrane attack complex in a cell membrane or it may associate with protein S to form a water-soluble analogue termed SC5b-C9 (118), which, in this thesis, is referred to as TCC. Thus, the levels of TCC reflect the degree of complement activation.

6.2 Papers II and III

6.2.1 Assessing causality using observational data

To assess the causal effect of donor age on recipient outcome, the most conclusive scientific method would probably be an experimental design where a large number of recipients were randomized to either receiving an organ older or younger than the recommended donor age criterion (119). This would randomly distribute the potential confounders between the two groups. However, this type of experimental design is not feasible for practical, and perhaps ethical, reasons. The solution to this problem could be to use observational data with a study design aiming to minimize the bias created by confounding. One way to minimize confounding is to include potential confounder variables in a regression model (e.g. Cox regression). Several methods have been used in previous studies to identify confounders. Some approaches have been solely data-driven (e.g. step-wise inclusion or exclusion methods), but it can be shown that some of these methods are conceptually flawed when the objective is to assess causality (120). For example, inclusion of so-called colliders can, as a result of selection bias, result in false associations between the variable of interest and the outcome variables, and thus lead to biased results (121).

A increasingly commonly used method to assess the structure of causality is the DAG. A DAG is a graphical model illustrating the hypothesized causal relations between a set of entities of interest. An arrow is drawn between variables A and B when the null hypothesis is that there is a causal effect of A on B. Thus, if the arrow is omitted, one makes a strong claim that there is no effect of A on B (121). In this work, a DAG was constructed to identify confounding and adjust for relevant covariates in the Cox-regression model. The DAG used in Paper II is shown in Figure 6.2. Notably, recipient urgency is a concept that is not directly measurable. In this work, we used life support and Scandiatransplant urgent status as surrogates for recipient urgency. However, as these surrogates do not plausibly capture all aspects of recipient urgency, there may still be some remaining confounding in our model. This is an example of the limitations of using observational data to assess causality. Nonetheless, we consider life support and Scandiatransplant urgent status to be good surrogates for urgency, and thus believe our model still has value.

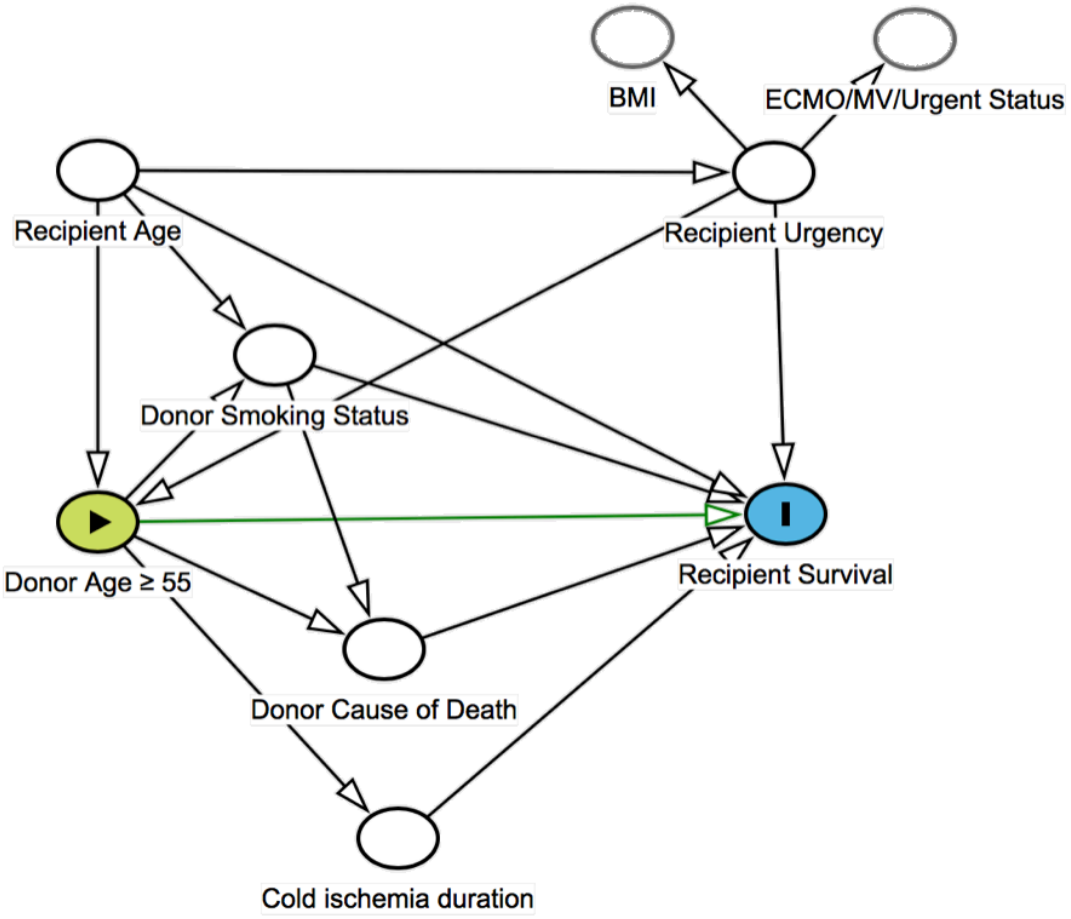

Figure 6.2. Simplified directed acyclic graph (DAG) used in Paper II. ECMO, extracorporeal membrane oxygenation; MV, mechanical ventilation.

6.2.2 Survival analysis

Survival analysis is a class of statistical methods applied to a set of data where the outcome variable is the amount of time until a specific event, often referred to as failure, occurs (122). In this work, survival analysis is applied to lung transplant recipient survival times and graft survival times after LTx. The distribution of failure times for the transplanted population is not known. Thus, only methods that do not make assumptions about this distribution are used (i.e. non-parametric or semi-parametric methods) in this work. Survival analyses are often complicated by censoring and truncation. In this work, right censoring is present as many subjects are still alive when the observation period ends (i.e. late 2017). As all patients are

observed from time of transplantation, left censoring is not present. All patients are included regardless of survival time, and thus truncation is not present.

6.2.2.1 The Kaplan-Meier estimator and the log-rank test

The Kaplan-Meier estimator is a non-parametric estimate of the survival function S(t) introduced by Kaplan and Meier. It is defined as:

$$\hat{S}(t) = \prod_{j|t_j \leq t} \left(\frac{n_j - d_j}{nj} \right)$$

where $\hat{S}(t)$ is the estimated survival at any given time t, n_j is the number of subjects at risk at time t_j and d_j is the number of failures at t_j (122). The Kaplan-Meier estimator is used in article II and article III to produce Kaplan-Meier plots with survival time t on the x-axis and the Kaplan-Meier estimator $\hat{S}(t)$ on the y-axis. In these plots, right censoring is indicated by a thin vertical line above the graph line. Survival estimates can also be calculated for different groups and presented on the same plot.

The log-rank test is a non-parametric test used to test the equality of hazard functions for two or more groups. Conceptually, contingency tables with number of survivors and number of failures for each group of interest are constructed at each distinct failure time. The information in these contingency tables is combined to create the test statistic. Thus, the log-rank test does not compare the groups at a specific time point, but takes the entire follow-up period into consideration. The log-rank test assumes that censoring is unrelated to prognosis. Moreover, it is most powerful when hazard rates between groups are proportional (122) (i.e. hazard ratios remain constant over time). In this work, we assume that censoring is unrelated to prognosis.

6.2.2.2 Cox regression

Cox regression is a commonly used semi-parametric survival analysis method introduced by Cox. It can be used to assess the association between one or more covariates (independent variables) and survival (dependent variable). The Cox regression model is given by:

$$h(t|X) = h_0(t)e^{X_1\beta_1 + \cdots + X_k\beta_k}$$

where $h(t|X)$ is the hazard function given a set of covariates X, $h_0(t)$ is the baseline hazard (the hazard if all covariates were zero), $X_1, \ldots, X_k$ are the covariates and $\beta_1, \ldots, \beta_k$ are the covariate coefficients. Importantly, Cox regression makes no assumption about the form of the baseline hazard, but assumes a parametric form of the effect that covariates have on the hazard rate. Specifically, the Cox model assumes that covariates have a multiplicative effect on the hazard and that this effect is constant over time (for all values of t). This is commonly referred to as the proportional hazard assumption. There are several ways to test the proportional hazard assumption. In this work, scaled Schoenfeld residuals were used. Furthermore, as with the log-rank test, Cox regression assumes non-informative censoring (122).

7 Main Results

7.1 Paper I

In total, 34 multi-organ donors were included in the study. When compared to healthy, living individuals, the following biomarkers were increased before the procurement operation: G-CSF, IFN-γ, IL-1ra, IL-4, IL-6, IL-7, IL-8, IL-10, IP-10, MCP-1, MIP-1β, PDGF, RANTES and TNF ($p< 0.001$). In contrast, the following biomarkers were below detection limit for most samples and thus excluded from further analyses: IL-1β, IL-2, IL-5, IL-9, IL-12, IL-13, IL-15, IL-17, eotaxin, bFGF, GM-CSF, MIP-1α and VEGF. Notably, the pre-operative cytokine levels were not related to cause of death, donor age, donor BMI, donor gender or donor time on ventilator.

The following biomarkers increased during procurement: TCC, IL1-ra, IL-6, IL-8, IL-10, IP-10, G-CSF, MCP-1, MIP-1b and PDGF (Table 7.1). The TNF/IL-10-ratio significantly decreased during surgery ($p<0.001$).

Biomarker	**Increase during surgery (Corrected) Median (IQR)**	**p-value**
TCC	1.3 (0.9 – 2.0)	0.007*
IFN-γ	0.6 (0.4 – 2.3)	0.264
TNF	0.8 (0.4 – 1.4)	0.275
IL-1ra	1.3 (0.9 – 3.4)	0.037*
IL-4	0.9 (0.3 – 1.8)	0.270
IL-6	2.3 (1.6 – 4.6)	0.003*
IL-7	1.1 (0.8 – 1.8)	0.209
IL-8	2.0 (1.1 – 2.9)	<0.001*
IL-10	3.5 (1.7 – 10.2)	<0.001*
IP-10	1.6 (0.9 – 3.0)	0.004*
G-CSF	1.7 (1.2 – 2.8)	<0.001*
MCP-1	3.2 (1.4 – 6.6)	<0.001*
MIP-1b	1.4 (1.0 – 1.9)	0.003*
PDGF	2.2 (0.5 – 13.0)	0.049*
RANTES	1.2 (0.6 – 2.2)	0.286

Table 7.1. Median fold change and interquartile range (IQR) of circulating biomarkers before multi-organ procurement (T0) compared with after free-preparation of thoracic organs (T3). P-values calculated using Mann-Whitney U-test and Wilcoxon matched-pairs signed rank test.

7.2 Paper II

In total, 913 patients underwent primary BLTx (excluding Re-Tx and multi-organ Tx) in the time period 2000 – 2013 at the five Scandiatransplant centers that perform LTx. Of these, 18.1% had CF, 40.4% had COPD, 25.4% had ILD and 16.1% had other diagnoses. Median donor age was 60 years (IQR 57- 64) in the older donor group (donors ≥55 years) and 41 years (IQR 26 – 48) in the younger donor group. For the overall population, recipient and donor age were significantly correlated (ρ=0.276, p<0.001). This was also evident among patients with COPD (ρ=0.188, p<0.001), ILD (ρ=0.238, p<0 .001) and in the "Other" group (ρ=0.390, p<0.001), but there was no significant correlation between donor and recipient age among patients with CF (ρ=0.059, p=0.451). Patients with CF and ILD receiving older organs were more commonly on life support (ECMO and/or MV) intended as bridge to transplantation compared to those receiving younger organs (CF: 31% vs. 8.1%, p=0.003; ILD: 24.6% vs. 6.9%, p=0.001). CF patients receiving older organs were also more commonly listed as urgent in the Scandiatransplant international organ exchange system (26% vs. 5.2%, p=0.006).

Median initial ICU LOS following LTx was 3 days (IQR 2 – 7.5) and ranged 0-97 days. In recipients with CF, the utilization of donors ≥55 years was associated with longer ICU LOS (3 vs. 5 days, p=0.034), but the ICU LOS was not significantly correlated with recipient age (ρ=0.057, p=0.480). Also, in recipients with ILD the utilization of donors ≥55 years was associated with longer ICU LOS (5 vs. 6.5 days, p=0.018), and although the correlation coefficient for recipient age and ICU LOS was higher than in the CF group, it did not reach significance (ρ=0.128, p=0.061). There were no significant differences in ICU LOS between recipients receiving organs ≥55 years and younger organs for the COPD (3 vs. 3 days, p=0.102) or the "Other" subgroups (5 vs. 4.5 days, p=0.806).

In the overall study population, 90-day, 1-year and 5-year survival were 94.7%, 86.5% and 67.6% respectively. There was no significant difference in overall survival (p=0.278) when comparing the utilization of donors ≥55 with younger donors. In multivariate models (as described in methods), the utilization of donors ≥55 was not associated with survival when donor smoking was excluded (Hazard ratio (HR) 1.0, 95 % confidence interval (CI) 0.8 – 1.4,

p=0.851) or included (HR 1.0, 95 % CI 0.7 – 1.4, p=0.981). In subgroup analyses however, recipients with CF receiving organs from donors ≥55 years had inferior survival in multivariate models with donor smoking status excluded (HR 4.0, 95% CI 1.7 – 9.2, p=0.001) or included (HR: 5.0, 95% CI: 1.8 – 14.1, p=0.002). For all other diagnoses, subgroup analyses did not reveal any difference in survival with the use of organs from donors ≥55 years compared to the use of organs from younger donors.

7.3 Paper III

In the period after the implementation of the ScULAS (1.5.2009 – 31.12.2014), 1,023 patients were listed for LTx, 71 (6.9%) were listed as urgent, 772 (75%) patients were transplanted, 102 (10%) died on the waiting list, 41 (4.0%) were permanently withdrawn and 108 (11%) were still waiting at the end of the period. The average utilization of available urgent calls during the study period varied from 71% to 94% (all-center average 84%) between centers. Furthermore, the average amount of time where all urgent calls were used varied from 0.7 to 4.1 months per year (all-center average 2.1 months per year) between centers during the study period. Patients listed as urgent were younger (40 years vs. 54 years, p<0.001). There were no significant differences in gender, height, pTLC or blood type. Furthermore, patients in the urgent cohort were less likely to have a negative panel reactive antibody (PRA) tests (66% vs. 81%, p=0.005). There were very few patients with obstructive lung disease (2.8% vs. 42%, p<0.001), and a higher proportion of patients with suppurative (30% vs. 13%, p=0.001) and other diagnoses (9.9% vs. 4.2%, p=0.038) on the urgent list compared to the regular list. While 39% of the urgent list had restrictive lung disease, only 28% of the regular list had (p=0.057). Patients listed as urgent were more commonly on ECMO (45% vs. 2.3%, p<0.001) or MV (9.9% vs. 2.1%, p=0.002) compared to patients listed as regular. Among transplanted patients, there were no differences in donor age (51 vs. 49 years, p=0.364) between patients listed as urgent compared to regular, but there was a trend towards a lower proportion with a positive donor smoking history (18% vs. 35%, p=0.055) in the urgent group. Furthermore, ex-vivo lung perfusion (EVLP) was only used in two cases in the urgent group. Not surprisingly, a higher proportion of the patients listed as urgent were transplanted during the first year after listing compared to those listed as regular (90% vs. 27%, p<0.001). While 81% of those listed as urgent were transplanted within four weeks, 86% within eight weeks and 89% within 12 weeks, the corresponding proportions for those with regular status were 4.3%, 5.9%, and 7.6% respectively. Furthermore, a higher proportion of the urgent status patients died or were

permanently withdrawn during the first year after listing (9.9% vs. 3.6%, p=0.020) compared to the regular waiting list. The patients who died on the urgent list were all women, five were on life support, four were listed for ReTx, and four had blood type O. In total, 15 patients died on the waiting list while on life support. Of these, 10 had not received urgent status, although available in all but one case. Importantly, having urgent status significantly increased the chance of being transplanted for patients on life support (p=0.026).

When comparing patients listed as urgent with patients listed as regular, 30-day graft survival (90.6% vs. 96.3%, p=0.042) and 90-day graft survival (87.5% vs. 94.5%, p=0.048) were significantly inferior among patients listed as urgent, but there were no differences in 1-year graft survival (81.3% vs. 85.5%, p=0.361) or overall graft survival (p=0.705). However, the overall graft survival must be interpreted carefully as our analysis was unable to detect a hazard rate below 1.6 with 80% power due to the low number in the urgent group. When exclusively analyzing patients without life support, we found no differences in 30-day graft survival (96.8% vs. 97.1%, p=0.612), 90-day graft survival (93.6% vs. 95.5%, p=0.649), 1-year graft survival (93.6% vs. 86.2%, p=0.415) or overall graft survival (p=0.212). Moreover, for all patients listed after introduction of ScULAS, we found a significantly lower graft survival in those on life support compared to patients not treated with life support (p=0.020). There was no difference in graft survival in the patients on life support when comparing those with and those without urgency status (p=0.377).

When comparing the pre- and post-implementation periods, we found an increase in the average number of transplants from 113/year to 136/year (+20%) while the average number of new patients listed increased from 141/year to 161/year (+14%). The donor utilization rate changed from 29.5% to 31.7% (+7%). Consequently, a significantly higher proportion of listed patients were transplanted (75% vs. 67%, p<0.001) and a significantly lower proportion died or were withdrawn from the waiting list (18% vs. 14%, p=0.041). Moreover, in the latter period, the waiting list included a higher proportion of patients with restrictive diseases (22% vs. 29%, p<0.001) and a lower proportion with obstructive diseases (48% vs. 39%, p<0.001) and “other” diseases (7.2% vs. 4.6%). Similar changes were observed among those who were transplanted, with a reduction in the proportion of LTx recipients with obstructive lung disease and an increase in LTx recipients with restrictive lung disease in the post-

implementation period. Furthermore, there was an increase in the proportion of patients who were bridged to transplantation using life support.

8 Discussion

8.1 Paper I

This study indicates that the surgical trauma associated with organ procurement increases systemic inflammation from an already elevated level in solid organ donors. These findings are in line with other studies that have assessed the inflammation associated with surgical trauma (123). However, the multi-organ procurement operation may be different from other surgical procedures in several ways. First, it involves extensive tissue trauma as it involves both a sternotomy and laparotomy, and meticulous free-preparation of organs over several hours. Furthermore, at the time of procurement, there may have been several days since the initial injury, and brain death-associated inflammation may already have persisted for a long period of time. To the best of our knowledge, no other study has assessed the inflammation associated with organ procurement in traditional organ donors.

8.1.1 Interpretation of plasma biomarkers

Notably, several inflammatory biomarkers were elevated before the procurement operation and increased during surgery, while others were not. This raises the question of whether this biomarker pattern can give clues to the underlying quality of inflammation and suggest ways to attenuate this process, which, in other studies, has been shown to be detrimental for the organ recipients (124). However, the interpretation of the pattern of plasma inflammatory biomarkers in organ donors is challenging for several reasons. First, the measured cytokines likely have multiple sources. Plausible sites of production are the dying brain, other injured parts of the body, and the ventilator-treated lungs (45). Circulating cytokines may, in turn, stimulate additional cytokine secretion in target cells located elsewhere in the body (125). The measured cytokines will thus be a mix of cytokines from several different production sites and they may be primary or secondary to the ongoing inflammatory process. Second, the mode of death and the associated injuries and the duration between the initial cause of death and blood sampling varied greatly between donors. It is possible that these factors influenced the levels of circulating biomarkers, although no statistical associations were found in our relatively small study population. Third, circulating cytokines have relatively short, but different, half-lives. For instance, TNF has a median half-life of 17 minutes (126), while IL-6 is known to be stable in circulation for longer periods of time (127). For many inflammatory biomarkers, knowledge about half-lives is limited. Thus, the levels of measured cytokines are not only

dependent on secretion from different production sites, but also on in-vivo half-lives. Taken together, this indicates that the plasma biomarkers profiles measured in our population of organ donors should be interpreted very carefully. However, in spite of these limitations, we believe that our study may still provide some insights of the quality of the inflammation in traditional organ donors.

8.1.2 Complement activation

Our study demonstrates that the complement system is activated in BDD and indicates that this activity increases during procurement. This is in line with other studies that have shown activation of the complement system in BDD before procurement. Notably, complement inhibitors have shown to improve graft quality in animal studies (128). However, to the best of our knowledge, there are no on-going clinical trials of complement inhibitors in human donors. Our study suggest that in future studies complement inhibition should not only be given during donor management in the ICU, but also during the procurement operation.

8.1.3 Release of pro- and anti-inflammatory cytokines

First, we found that IL-6 was elevated before procurement and increased significantly during surgery. IL-6 is known to be secreted in response to the activation of toll-like receptors (TLR) by danger associated molecular patterns (DAMP) seen in BDD before procurement (129,130). In addition, surgical trauma has been shown to markedly induce secretion of IL-6 (130). IL-6 is considered to have both pro- and anti-inflammatory properties (131), but its significance in BDD not well known. Interestingly, Murugan et al. found that high circulatory levels of IL-6 in the donor were associated with reduced hospital-free survival in organ recipients (132). However, it is unclear whether this association is due to deleterious effects by IL-6 itself or if IL-6 is the byproduct of an unfavourable inflammatory process in the donor. To the best of our knowledge, studies using IL-6 inhibitors such as Tocilizumab in human BDD have not been published.

Second, similar to IL-6, we found that levels of IL-10 and IL-1ra were elevated before procurement and increased during surgery. IL-10 is known to have anti-inflammatory properties, and inhibit the secretion of many other pro-inflammatory cytokines such as TNF

(133). Similarly, IL-1ra inhibits anti-inflammatory effects by antagonizing the IL-1-receptor (134). Notably, we observed a shift towards a lower TNF/IL-10-ratio during the procurement operation. Some authors have argued that the TNF/IL-10 ratio can be used as a measure of the balance between pro-inflammatory and anti-inflammatory activity (135,136). These findings could be a result of the methylprednisolone that is routinely administered to organ donors before procurement. However, it is not certain whether this ratio is meaningful when measuring plasma levels of these cytokines in BDD. As mentioned above, plasma levels is the sum of cytokine production at several production sites and the half-lives may differ between cytokines. Thus, it is not certain that the observed shift in plasma TNF/IL-10 necessarily reflects a shift towards anti-inflammatory activity at the sites of cytokine production. Nevertheless, one could argue that the change in plasma TNF/IL-10 represents a shift towards a more systemic anti-inflammatory milieu. Nonetheless, the TNF/IL-10-ratio only represents one dimension in a very complex system, and the change in other mediators should probably also be considered to assess the total inflammatory state of the donor.

Third, we found that the secretion of several other biomarkers was increased during surgery. These were IL-8, IP-10, G-CSF, MCP-1, MIP-1b and PDGF. IL-8 is a chemokine known to induce chemotaxis in neutrophil granulocytes, and also activate other cells of the immune system (137). Similarly, IP-10 is a chemokine that primarily induces chemotaxis in lymphocytes (138), while MCP-1 is a chemokine that principally induces chemotaxis in monocytes (139). Furthermore, MIP-1b is known to have chemotactic properties and induce pro-inflammatory responses (140). G-CSF stimulates the bone marrow to produce and release granulocytes into the circulation (141). Finally, PDGF is a growth factor relevant in wound healing (142), and its relevance in the setting of organ donor inflammation is uncertain. Taken together, the increase in these pro-inflammatory biomarkers indicates an increase in systemic inflammation during procurement.

8.2 Paper II

To the best of our knowledge, this is the first multi-center study to assess the effects of donor age in a Scandiatransplant cohort. The findings in this study may improve our understanding of the effect of using older donors in LTx. First, our results indicate that there are no significant differences between recipient survival with donors older than the standard donor criteria (≥55 years) and younger donors for recipients with COPD, ILD and other diagnoses. However, recipients with CF had inferior survival when receiving lungs from donors ≥55 years. Second, this study demonstrates that the use of older donors' lungs was associated with longer ICU LOS in patients with ILD and CF. Third, age-matching was evident in recipients with COPD, ILD and other diagnoses, but not in recipients with CF.

8.2.1 Recipient Survival

To the best of our knowledge, this is the first study to indicate that the use of older donors results in inferior survival in patients with CF, while no such negative effects are seen in recipients with other diagnoses. Several other studies have investigated the consequences of using older donors in LTx with conflicting results. Importantly, the majority of these studies have focused on the overall lung transplant population and not subgroups. Notably, the cohorts in these studies differ substantially in terms of donor characteristics, transplant type and recipient characteristics. It is conceivable that this might explain some of the observed discrepancy between previous works on this subject.

One early study by Novick et al., using a cohort (n=5052) of both SLTx and BLTx performed between 1987 – 1997, found that donor age >50 was associated with reduced survival, and that this association increased if combined with long ischemia times (143). This study differs from our work by suggesting that the use of older donors negatively affects all transplant recipients. However, there are several differences between our cohort and the cohort in the work by Novick et al. that may account for this inconsistency. Primarily, this is an old cohort including recipients with generally lower survival than those commonly seen in transplant recipients today, probably due to candidate selection, improvements in surgical techniques and follow-up. Moreover, the cohort include a large proportion of SLTx. In contrast to the study by Novick et al., later studies by Bhorade et al. (144), Thabut et al. (145), Fischer et al. (86), and Dahlman et al. (146) found no negative effects of using older donors. However,

none of these studies focused on recipient subgroups, and, for the overall transplant population, their conclusion is similar to ours. In 2007, DePerrot et al. examined the effect of using donors >60 years, and found that the short-term outcome was similar, but that older donors resulted in more BOS and reduced 10-year survival (147). Three years later, Pizani et al. found that the use of donors >55 years resulted in more BOS, but did not find any difference in short- or long-term mortality (87). None of these studies focused on recipient subgroups, but their cohorts are more comparable to ours as they contain less SLTx and more BLTx. Interestingly, the study by Pizani et al., which could not identify any inferior effects of using older donors, only included one CF recipient who received an older organ, while, in the study by DePerrot et al., 13 (22%) of the recipients receiving older organs had CF. In 2013, a large study by Bittle et al. including 10,666 patients found that the use of organs ≥65 years resulted in inferior survival, but the use of organs aged 55 to 64 years yielded a similar outcome as when using younger organs (148). This study did not focus on recipient subgroups, and only 7% of the patients receiving organs from donors aged 55 to 64 had CF. It is therefore possible that a negative effect of older donors in CF recipients could have been missed. Furthermore, almost half of the transplantations in the cohort were SLTx, reducing the comparability to our work. Recently, Sommer et al. examined recipient outcome in 27 patients who received selected organs >70 years, and found no difference when comparing these to patients receiving younger organs (23). All of these transplantations were BLTx, but none of the recipients receiving older organs had CF. Similarly, Hecker et al. found no difference in recipient survival when using selected organs 60-69 years and organs >70 years compared to younger organs, but the older donor groups contained very few recipients with CF (149). Furthermore, Holley et al. found no negative effects of using donors >55 years, but only three recipients receiving older donors had CF (150). Recently, other studies similar to ours have focused on whether older donor organs could have negative effects in certain recipient subgroups. Shigemura et al. found that recipients with PHT or prolonged cardiopulmonary bypass time had inferior survival when receiving organs >55 years (89). Hayes et al. analyzed a cohort of 23,704 patients and found that the use of older donors in younger recipients resulted in inferior survival (88). As LTx recipients with CF are, in general, younger than recipients with other diagnoses, our findings are comparable to those by Hayes et al.

The reason why the use of older donors leads to inferior results in recipients with CF and not other diagnoses is not obvious. It is possible that donor-recipient age-mismatch plays a larger role in recipients with CF, as they generally are younger and have fewer comorbidities, so survival to a higher degree is determined by the vitality of the lung graft and not other causes of death. Another explanation could be that old lungs have increased susceptibility for the bacterial colonization of the upper airways seen in recipients with CF. Unfortunately, the data available to this study do not give further clues as to why CF patients seem to be the only group where donor age affects post-transplant survival.

8.2.2 Length of stay in intensive care unit

Notably, patients with CF and ILD had longer ICU LOS when receiving older donor lungs. However, for patients with ILD, it is possible that this association is partly due to effects of recipient age, as recipient age and donor age were moderately correlated. Notably, there was a trend of correlation ICU LOS and recipient age in patients with ILD, although this did not reach statistical significance. For patients with CF there was no correlation between recipient age and ICU LOS, suggesting that donor age might be the causal factor for the increased ICU LOS.

8.2.3 Age-matching

Even though there are no official protocols or guidelines to match donor and recipient age at the included centers, there was a significant correlation between donor age and recipient age for patients with ILD, COPD and in the "other" diagnoses group. This could reflect a sentiment among transplant physicians that organs from older donors should be allocated to older recipients. Notably, no such correlation was evident for patients with CF. It is possible that this is because the CF population is younger, and that it is difficult to allocate age-matched organs to these patients, especially if urgency increases. Supporting this hypothesis, we found that there was a significantly higher proportion of CF patients who were on life support as a bridge to TX, or listed as urgent in the ScULAS.

8.2.4 Limitations

This study has several limitations. First, it is a retrospective study with potential unmeasured confounders. Second, data were not available for relevant secondary end-points such as primary graft dysfunction, time on ventilator, acute rejection and spirometry measurements. Furthermore, some variables had missing data, and some subjects were therefore excluded from the multivariate analyses. Third, generalizability could be limited, as relatively few recipients were older than 65 years, and few were on life support with MV or ECMO at the time of transplant. Fourth, recipient urgency is a non-measurable factor that can lead to confounding because it may increase the chance of accepting an older organ and influence survival. In this study, the use of life support and ScULAS urgency status were used as surrogates. However, it is possible that not all aspects of recipient urgency are captured, and that some confounding still remains. Future studies with detailed information on recipient urgency would be necessary to clarify this question. Finally, whether a donor age of 55 years is the optimal cut-off value for recipients with CF was not tested in this study and needs to be explored in studies with larger study populations.

8.3 Paper III

This is the first article to describe the ScULAS and analyze the results of its implementation. First, this study gives an overview of the prioritization pattern resulting from clinical judgment in the ScULAS. Second, it demonstrates that even though the majority of patients on the urgent waiting list were transplanted very rapidly, the urgent waiting list mortality was higher than in the regular waiting list. Third, this paper shows that recipients from the urgent waiting list have inferior short-term graft survival compared to regular recipients, and that this potentially reflects the increased proportion of patients on life support in the urgent group. Fourth, this study demonstrates that the centers on average were out of urgent calls 2.1 months per year during the study period. Last, it shows that the implementation of ScULAS occurred simultaneously with an increase in number of transplantations and a shift towards listing more patients with restrictive disease and less patients with obstructive disease.

8.3.1 Patients given urgent status in the ScULAS

As mentioned in the introduction, there are no specific clinical criteria for urgent listing in ScULAS. Instead, each urgent listing is evaluated individually at each center using clinical

judgement. This study gives an overview of the pattern of prioritization resulting from clinical judgment in ScULAS. The purpose of this section is to view this pattern of prioritization with the ethical lenses of utility and equity and to compare it to other lung allocation systems.

This study found that patients with suppurative and likely also restrictive lung diseases were more commonly given urgent status in the ScULAS. Notably, patients with these diagnoses more frequently experience rapid deterioration (Personal communication: Are Holm). Similarly, this study also demonstrated a higher proportion of patients on life support on the urgent list compared to the regular list. As patients with rapidly progressing suppurative or restrictive diseases, and patients on life support, may have a poor chance of survival within the near future, one may argue that prioritizing these patients is in line with the ethical principal of equity as all patients in need of transplantation should have a comparable opportunity of receiving treatment (90). If patients with rapidly progressing diseases were not prioritized, they would most likely not have a realistic chance of receiving treatment as they would die while queuing for an organ. On the other hand, if the number of listed patients is higher than the number of transplantations and all patients on the waiting list gradually progresses towards death, transplanting a patient on the urgent list implies that another patient will not receive a transplant. This may imply that the patient who is not transplanted either slowly progresses towards death, or that the patient's condition worsens and is considered urgent at a later point. If the goal is to follow the principle of equity, the number of patients given urgent status must be balanced so that the chance of receiving treatment for a patient with critical lung disease and a patient with a lung disease with slower progression is comparable. Our study found that 7.4% of listed patients in the ScULAS received urgent status. In comparison, the HELTx system in France gave 14.2% of the transplanted patients urgent status (151). Whether the current number of urgent calls is optimal to achieve a fair allocation of lungs is not certain. It is also not certain whether it is optimal to dichotomize recipient urgency, so that the recipient is either considered urgent or not urgent. In the LAS, recipient urgency is reflected in a score from 0 – 100 (93).

Patients with obstructive diseases were rarely prioritized in the ScULAS. Although patients with obstructive diseases also may have exacerbations, which may imply a poor prognosis in the near future, in general, they do not seem to be granted urgency status in the ScULAS.

Patients in this group are generally older and may have more comorbidities, and thus post-LTx outcome might be presumed to be poorer than for patients with other diagnoses. Consequently, it may be reasonable to not prioritize this group from a utilitarian perspective, such as is seen in the ScULAS. However, from the perspective of equity where all patients should have a comparable chance of receiving treatment, one may question the fairness of not giving priority to this group. Although the ScULAS has no predefined criteria, it seems to prioritize in a similar manner to the HELTx system in which patients with COPD are excluded in the predefined criteria (95). In contrast, the SULAS in the UK does give priority to patients with COPD according to predefined criteria (152).

The ScULAS also seems to prioritize younger patients. From a utilitarian perspective, this may be reasonable, because younger patients may have less comorbidities and thus a better post-TX prognosis. Moreover, one may argue, from a perspective of equity, that all humans should have a comparable chance of having a normal lifespan (90). Thus, it may be more reasonable to prioritize a person who is young and has experienced less of life than a person who is older and is closer to achieving a normal lifespan. In the UK, the SULAS criteria gives priority to patients under 16 years of age (152). Similarly, the HELTx seems to prioritize younger patients (96), although not formalized in its urgency criteria. In contrast, in the US, the implementation of LAS have been associated with higher mean age in the transplant population (94).

There was a higher proportion listed for ReTx on the urgent waiting list compared to the regular waiting list. In total, 9.9% of urgent patients in ScULAS were listed for ReTx. From an utilitarian point of view, this can be questioned as patients receiving ReTx having inferior survival compared to patients receiving first-time transplantations (18). However, from an egalitarian point of view where all patients should have the same chance of attaining good health, one may argue that whether the patient is previously transplanted or not is irrelevant (90). Notably, at this point, the ScULAS is different from HELTx and SULAS where patients listed for ReTx are not given urgent status according to the predefined criteria (95,152).

8.3.2 Time to transplantation and waiting list mortality

Not surprisingly, the supra-national priority given to patients with urgent status resulted in very short waiting times in this group. Of those in the ScULAS, 81% were transplanted within 4 weeks. However, the waiting list mortality was also higher in the urgent vs. the non-urgent group. This indicates that organ shortage is also relevant for the limited number of patients given urgent status, and that efforts to expand the donor pool may be beneficial also for this group.

In contrast to the HELTx system where a patient may be listed as urgent for maximum 14 days (95) (exceptions are allowed in special cases), there is no limit for how long a patient can be listed as urgent in ScULAS (98). This might give individual centers an incentive to list as urgent not necessarily those with short expected survival, but also those who may be expected to have a particularly long waiting time on the national regular list, such as patients with combinations of blood type and height that are uncommon in the donor pool. Importantly, we saw no overrepresentation of such patients among the urgently listed, although the proportion with negative PRA was slightly lower in the urgent group. Furthermore, only about half of the urgent patients were transplanted after two weeks, indicating that a time limit similar to that in the HELTx might not be suitable in this system.

In total, two thirds of patients on life support who died on the waiting list did not have urgent status. The reasons for this are unclear, but notably urgent status was available in all but one case. However, some might suspect that a desire to economize the limited number of urgent calls in order to save more lives influenced the decision-making process. As having urgent status increased the chance of receiving a transplant among patients on life support, one might argue that urgent status should be granted by predefined criteria to ensure equality between these patients. On the other hand, detailed information in each individual case was not available in this study, and should preferably be analyzed before making changes to the ScULAS.

8.3.3 Outcome of the patients given urgent status

There was inferior short-term graft survival in recipients given urgent status, and, although there was no significant difference in overall graft survival, our statistical power to detect such a difference was limited. It is likely that the inferior short-term graft survival in the urgent group was due to the higher proportion with life support in this group, as there was no difference when patients without life-support were analyzed separately. This is in line with other studies that have shown that the use of life support is associated with inferior survival (153). From an utilitarian perspective, one may thus question whether the prioritization in ScULAS is reasonable as the patients who are given priority have inferior graft survival (90). However, a more relevant question might be whether the true survival benefit is inferior in the urgent group. Specifically, this relates to how many extra days are added to an individual's life by receiving a lung transplant. This is a methodologically difficult question to answer. Nonetheless, even though the post-TX survival in the urgent group is lower than in the non-urgent group, it is not certain that the true survival benefit in the urgent group is inferior, as these patients have a poor prognosis in the very near future.

One could speculate that the inferior outcome seen in urgent patients was because of a tendency to accept lower-quality donors' lungs as an attempt to save critically ill patients. However, there was no evidence of increased use of marginal donor lungs in patients considered urgent. There was no difference in donor age, and the proportion with a positive donor smoking history tended to be lower in the urgent group compared to the regular group. Furthermore, EVLP was only used in two cases in the urgent group.

8.3.4 Utilization of ScULAS

The utilization of ScULAS varied between centres. Notably, the member centres did not use all of the urgent calls that were available. This might indicate that the centres seemed to honor the intent of the system by not maximizing their own benefit and using urgent calls when not strictly necessary. On the other hand, the centers were out of urgent calls on average 2.1 months per year. From a perspective of equality, this may be a concern as the fourth urgent patient would not receive priority even though he or she is in the same situation as the third urgent patient who received the last urgent call that calendar year. One could argue that this speaks towards increasing the number of urgent calls. However, in spite of an increase, a

fixed number of urgent calls could still result in periods where centres are out of calls, and thus the problem of inequality remains unsolved. Another solution would be to remove the limit, but this may also be problematic as it may affect the threshold for giving a patient urgent status. Moreover, removing the limit may lead to inequalities between the member countries, as different listing practices may give rise to different demand of urgent calls. Thus, some countries may end up as net exporters of donor organs while others are net importers. While the introduction of predefined clinical criteria, such as in the LAS, HELTx and SULAS could be one solution that would improve equality between listed patients, it would not necessarily ensure equality between member countries in Scandiatransplant.

8.3.5 Changes in the lung transplant population after implementation of ScULAS

Several changes that occurred simultaneously with the implementation of ScULAS. First, the number of transplantations increased, which may be the main reason for the decrease in wait list mortality. Second, there was a shift towards listing more patients with restrictive diseases and less patients with obstructive diseases. Additionally, the number of patients on life support increased. While it is not obvious that the ScULAS influenced the preference for restrictive over obstructive patients, the implementation of ScULAS may have encouraged the listing of patients who were previously considered too ill to be listed for transplantation as the waiting time would be too long. In particular, it may have reduced the barrier for using life support as a bridge to transplantation.

8.3.6 Limitations of this study

This study has several limitations. First, it is a retrospective, registry-based study. Second, the number of urgent patients is small which limits the power of statistical analyses. Third, the introduction of the urgency system occurred in the middle of a general increase in the number of lung transplants performed. This may preclude conclusions about the effects of the allocation system on the overall performance of the lung transplant activity, particularly concerning waiting list survival. Last, certain clinical data (e.g., FEV1 and 6MWT) may have been less available in the urgent cases, leading to an ascertainment bias.

9 Conclusions

9.1.1 Paper I

- The elevation of several inflammatory biomarkers in plasma indicates that there is systemic inflammation in BDD before procurement
- The secretion of inflammatory biomarkers into circulation increases during organ procurement
- As a wide array of inflammatory biomarkers are elevated and increase during the procurement operation, the systemic inflammation seen in organ donors likely involves several segments of the immune system including the complement system

9.1.2 Paper II

- The use of organs from donors older than the standard donor criteria (≥55 years) results in inferior survival in recipients with CF when comparing to the use of organs from younger donors
- For recipients with COPD, ILD or in the "other" diagnoses group, there were no differences in survival when comparing the use of organs from donors older than the standard donor criteria (≥55 years) and organs from younger donors
- The use of organs from donors older than the standard donor criteria (≥55 years) was associated with longer ICU LOS in recipients with CF and ILD when comparing to the use of organs from younger donors

9.1.3 Paper III

- The urgent waiting list had a higher proportion of patients with suppurative lung disease, younger patients and patients on life support than the regular waiting list. Patients with obstructive lung disease were rarely given urgent status
- Patients listed as urgent received organs substantially faster than patients on the regular list
- Waiting list mortality was higher for patients listed as urgent compared to patients listed as regular
- Patients listed as urgent had inferior short-term survival, and this was likely because of the higher proportion of patients on life support on the urgent list

- Although the ScULAS had no predefined criteria, the member centers did not use their maximum number of urgent calls in the study period. However, member centers were out urgent calls on average 2.1 months per year
- The implementation of the ScULAS was associated with a shift towards listing more patients with restrictive diseases, more patients on life support and less patients with obstructive diseases

10 Clinical implications and future perspectives

Hopefully, this thesis adds insights to the field of organ transplantation that can benefit patients with end-stage organ disease. Specifically, we believe this work may have implications for three important high-level challenges in organ transplantation:

1. Can we increase the numbers of donor organs that can be used for transplantation?
2. Can we ensure fair and efficient allocation of donor organs?
3. Can we improve recipient outcome after transplantation?

10.1.1 Paper I

This papers shows that there is systemic inflammation in the organ donor before procurement which is augmented during the procurement operation. These results might imply that efforts to reduce inflammation in the donor should not only be sought before procurement, but also during the procurement operation or after procurement using *ex-vivo* perfusion systems. Furthermore, our results give clues regarding potential targets to reduce inflammation. For example, our findings suggest activation of the complement system before and during the organ procurement. Further studies should evaluate whether treatment to reduce complement activation before and during procurement may improve recipient outcome. Moreover, our results show that a wide range of circulating inflammatory biomarkers and inflammatory mediators are increased before and during procurement. As an increasing number of targeted anti-inflammatory drugs are available, further studies should evaluate whether these could reduce donor inflammation and thus increase the number of organs that can be used for transplantation and improve recipient outcome.

10.1.2 Paper II

This paper shows that patients with CF have inferior survival when receiving an organ older than the recommended criteria. This result is especially relevant for patients with CF that are sufficiently clinically stable to wait for another organ if the organ available is older than in the donor criteria. As using older donors does not seem to impact survival for patients with other diagnoses, improved allocation of older organs could result in an overall improved recipient outcome. Future studies should seek to replicate these findings, and also explore other relationships between donor characteristics and recipient outcome that could further optimize

organ allocation. Ideally, if the Scandiatransplant database were improved to avoid missing data and include more donor and recipient parameters, then advanced allocation algorithms, continuously improved by new data added, could optimize allocation according to knowledge about relationships between donor characteristics and recipient outcome.

10.1.3 Paper III

This paper describes the characteristics of the patients who are prioritized in the ScULAS and demonstrates the mechanics of this system. Our findings may increase the transparency of the ScULAS for member centers, collaborating institutions and the general population. Notably, to the best of our knowledge, no previous publications about ScULAS are available except the general guidelines (98). Our study might also be useful to refine and improve the ScULAS in the future, and thus ensure fair and efficient allocation of donor organs.

11 References

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