Ex Vivo Lung Perfusion

Clinical and experimental studies

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For patients who are suffering from end-stage lung disease, lung transplantation is a life-prolonging therapy. The number of donor lungs is limited and the majority of available donor lungs, in some regions up to 85%, are discarded due to known or presumed organ dysfunction. Lung donations after cardiac death (DCD) and increased utilization of lungs from donations after brain death (DBD) could increase the availability of donor organs. *Ex vivo* lung perfusion (EVLP) is a method that has been developed for the preservation and evaluation of donor lungs during continuous perfusion and ventilation. EVLP is applicable to lungs harvested from DCD, as well as to the lungs of DBD with suboptimal lung function.

Method: In Papers I and II, an uncontrolled DCD situation was simulated in a pig model. The currently suggested protocol for DCD lung procurement, involving post-mortem administration of heparin followed by chest compressions and intrapleural cooling of the lungs to 12°C, was compared to a simplified procurement method that does not use heparin and employs a less-invasive cooling technique. In Papers III–V, the methods and results for EVLP for the salvage of initially rejected human donor lungs were investigated. Rejected donor lungs with inferior function were retrieved and connected to the EVLP system. Assessments of lung function, with respect to circulatory and respiratory parameters, were performed. EVLP-treated lungs that were deemed to have normal function were transplanted into recipients from the conventional waiting list for transplantation. The short-term and long-term outcomes for the recipients of the EVLP-treated lungs were compared to a consecutive series of patients who received non- EVLP lungs prepared according to the standard protocol.

Results: In Papers I and II, the lung function assessed during EVLP and at post-EVLP analyses in terms of the water content of lung tissues and markers of lung injury, did not differ significantly between the treatment and control groups. In Papers III–V, 25 pairs of rejected donor lungs underwent EVLP. Eighteen double lungs and four single lungs were transplanted after EVLP, and the recipients (EVLP group; N=22) were compared with recipients of conventionally prepared lungs (Control group; N=115). The median time to extubation (p=0.26) and the median stay in the intensive care unit (p=0.06) did not differ significantly for the two groups. Primary graft dysfunction higher than grade 1 was noted for 14% of the recipients in the EVLP group and 11% of the Control group at 72 hours post-transplantation. The cumulative 1-year survival rates were 89% for the EVLP group and 82% for the Control group. The cumulative survival rates for up to three years of follow-up were comparable for these two groups (p=0.67).

Conclusion: Papers I and II provide support for the notion that a no-touch period of 1 hour after death in cases of uncontrolled DCD would not compromise donor lung function. This would allow the next-of-kin to spend time with the deceased and to facilitate the making of a well-founded decision about organ donation. Papers III–V demonstrate that EVLP of initially rejected lungs from DBD can be safely performed and contribute to the lung transplantation program without compromising the outcomes for recipients. With the implementation of a well-functioning EVLP program, up to 50% of lungs from DBD multi-organ donors could be transplanted.

Key Words: Lung transplantation; Ex vivo lung perfusion; donation after cardiac death; donor lung procurement; lung disease.

List of publications

This thesis is based on the following papers, which in the text will be referred to by their Roman numerals (I–V).

I. Andreas Wallinder, Stig Steen, Hans Lidén, Christoffer Hansson, Aziz A. Hussein, Trygve Sjöberg and Göran Dellgren.
Heparin does not improve graft function in uncontrolled non-heart-beating lung donation: an experimental study in pigs.
European Journal of Cardiothoracic Surgery, 2013. 43(2):413–9.

II. Andreas Wallinder, Christoffer Hansson, Stig Steen, Aziz A Hussein, Trygve Sjöberg, Göran Dellgren.
A simplified preservation method for lungs donated after cardiac death.
Journal of Heart and Lung Transplantation, 2014. 33(5):528–35.

III. Andreas Wallinder, Sven-Erik Ricksten, Christoffer Hansson, Gerdt C. Riise, Martin Silverborn, Hans Lidén, Michael Olausson, Göran Dellgren.
Transplantation of initially rejected donor lungs after ex vivo lung perfusion.
Journal of Thoracic and Cardiovascular Surgery, 2012. 144(5): 1222–8.

IV. Andreas Wallinder, Sven-Erik Ricksten, Martin Silverborn, Christoffer Hansson, Gerdt C. Riise, Hans Lidén, Anders Jeppsson, Göran Dellgren.
Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study.
European Journal of Cardiothoracic Surgery, 2014. 45(1): 40–4; discussion 44–5.

V. Andreas Wallinder, Gerdt C. Riise, Sven-Erik Ricksten, Martin Silverborn, Göran Dellgren.
Transplantation after Ex-Vivo Lung Perfusion: an early follow-up
Manuscript.

Table of contents

Summary

Lung transplantation is a life-prolonging therapy for patients who are suffering from end-stage lung disease. Unfortunately, as the number of available donor lungs does not come close to meeting the demand in many countries, many patients die while awaiting transplantation. Strategies to increase the number of available donor lungs include donation after circulatory death (DCD) and extended use of lungs from donation after brain death (DBD). DCD was the prevailing donation strategy before the introduction of the brain death concept in the 1980´s. However, as a investigation of lung function is impossible in the DCD setting, there is a risk that injured donor lungs may be transplanted. *Ex vivo* lung perfusion (EVLP), which is a method for the preservation and evaluation of donor lungs during continuous perfusion and ventilation, has proven ability to distinguish reversible from non-reversible donor lung pathologies.

In Papers I and II of this thesis, the lung donation process for uncontrolled DCD is explored. In this scenario, the donation process is initiated after unsuccessful resuscitation of the patient in the emergency room. The organ procurement procedure must not only effectively protect lung function, but also be minimally invasive, so as to ensure acceptance of this form of organ donation among the public, the next-of-kin and hospital personnel. In a pig model, we simulated the uncontrolled DCD situation and investigated a simplified lung procurement regime. In Paper I, we show that post-mortem administration of heparin followed by chest compressions, does not significantly improve organ function when evaluated during EVLP. In Paper II, a procedure that involved post mortem, intrapleural cooling of the lungs to 12°C with four chest tubes and intermittent shifting of fluids was compared to a less-invasive technique with two chest drains and no shifting of fluids, which produced a milder hypothermia (23°C). As in Paper I, the lungs were evaluated by EVLP. The less invasive technique produced lungs that were functionally equivalent to those in the control group.

Taken together, Papers I and II provide support for a no-touch period of 1 hour after death, a period of time that would not compromise donor lung function but which would allow the next-of-kin to have some time with the deceased and to make a well-founded decision regarding donation. These papers also demonstrate that this procurement regime can produce transplantable lungs and could be used in the clinical setting.

A high percentage, in some regions up to 85%, of lungs from DBD are discarded due to known or presumed organ dysfunction. EVLP evaluation of initially rejected DBD lungs has been suggested as a method to recondition and assess the functions of' rejected donor lungs. In Papers III–V, we report on and discuss methods for EVLP and outline the results from the introduction of an EVLP program of initially discarded DBD lungs in our department at Sahlgrenska University Hospital, Gothenburg, Sweden. In a review of the first 11 EVLP-treated lungs that were transplanted into patients (Paper IV), both the time to extubation and the time spent in the intensive care unit (ICU) were longer for the patients who received EVLP-treated lungs than in patients who were transplanted with non-EVLP lungs prepared in the conventional way. These findings were not reproduced in Paper V, which reports on a larger cohort of transplant recipients. Over a period of 36 months, 294 donor lungs were offered to our center, 115 of which were accepted for transplantation and served as controls. In total, 25 pairs of rejected donor lungs were subjected to EVLP. Of these lungs, 18 double lungs and 4 single lungs were subsequently transplanted. The median time to extubation was 7 hours (range, 3–899 hours) in the EVLP group versus 6 hours (range, 2–1440 hours) in the control group (p=0.26). The median ICU stays for the EVLP and Control groups were 3 days (range, 1–39 days) and 2 days (range, 1–60 days), respectively (p=0.06). Primary graft dysfunction higher than grade 1 was present in 14% of the EVLP group and in 11% of the conventional group at 72 hours post-operatively. The duration of stay in the ICU still tended to be longer for the patients who received EVLP-treated lungs. Injuries to the lungs that occur while still in the donor are often accompanied by pulmonary edema, which, in our experience, is not completely resolved during EVLP, but rather in the recipient. Therefore, it is reasonable to expect that the patients in the EVLP group will spend more time on the ventilator and have longer ICU stays. We propose that EVLP provides a way to select potentially good lungs from among the initially rejected ones, although it does not *per se* reverse donor lung pathology, other than recruits atelectasis and removes secretions that obstruct the bronchi. In Paper V, the results of up to 3 years of follow-up of the patients are presented. The cumulative 1-year survival rate was 89% for the EVLP group and 82% for the Control group. The cumulative survival at up to 3 years of follow-up was also similar between the two groups (p=0.67). We conclude that EVLP evaluation of initially rejected donor lungs can be safely performed and can be a significant contribution to a lung transplantation program without compromising recipient outcomes. We believe that a well-functioning EVLP program would salvage up to 50% of lungs from multi-organ donors for transplantation.

Abbreviations

BOS	Bronchiolitis Obliterans Syndrome
CO	Cardic Output
COPD	Chronic Obstructive Pulmonary Disease
CPR	Cardio-Pulmonary Resuscitation
DBD	Donation after Brain Death
DCD	Donation after Cardiac/Circulatory Death
DLTx	Double-Lung Transplantation
ECC	Extra-Corporeal Circulation
ECMO	Extra-Corporeal Membrane Oxygenation
EVLP	*Ex Vivo* Lung Perfusion
HCT	Hematocrit
HLTx	Heart and Lung Transplantation
ICP	Intracranial Pressure
ICU	Intensive Care Unit
ISHLT	International Society of Heart and Lung Transplantation
LA	Left Atrium
LTx	Lung Transplantation
NHBD	Non-Heart-Beating Donor
P/F ratio	PaO_2/FiO_2 ratio
PA	Pulmonary Artery
PAH	Pulmonary Arterial Hypertension
PF	Pulmonary Fibrosis
PGD	Primary Graft Dysfunction
PVR	Pulmonary Vascular Resistance
RAS	Restrictive Allograft Syndrome
RBC	Red Blood Cell
SLTx	Single-Lung Transplantation
SU	Sahlgrenska University Hospital
W/D	Wet/Dry Ratio

Introduction

History of Lung Transplantation

The technical feasibility of the lung transplantation procedure was described during the 1940s and 1950s, and the first human lung transplantation was performed in Mississippi in 1963 by Dr. James Hardy (1). The transplanted patient, who survived for 18 days post-surgery, was later identified by the Miami News as the convicted murderer John Richard Russle. There followed a long series of failed attempts at achieving long-term survival after lung transplantation. The main factor that limited survival at the time was the absence of effective immunosuppressive drugs and a fully developed heart-lung machine.

Cyclosporine was discovered in Norway in 1969, and its immune-suppressive effects were revealed 3 years later. When Dr. Bruce Reitz performed the first successful heart and lung transplantation in 1981 (2), the breakthrough was largely due to the use of a cyclosporine-based immunosuppressive protocol. In 1983 and 1986, Dr. Joel Cooper reported the first successful single-lung and double-lung transplantations that achieved long-term survival of the recipients (3, 4).

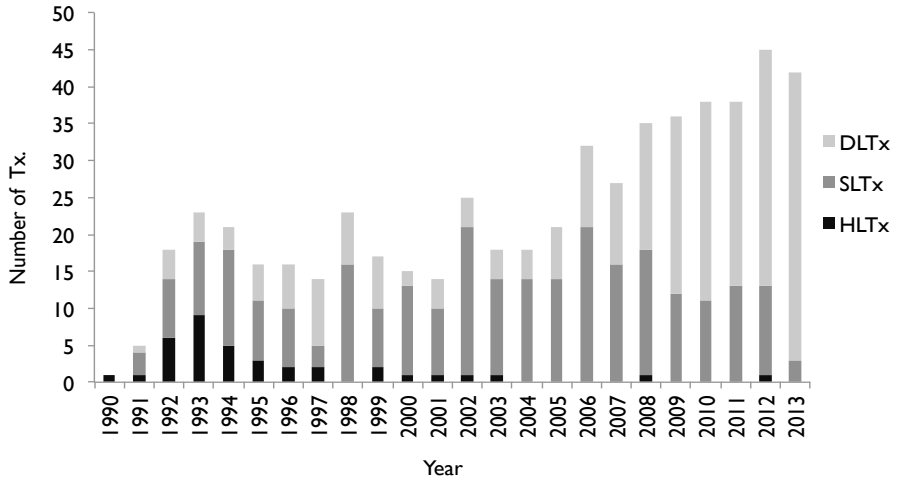
Lung transplantation today

The International Society of Heart and Lung Transplantation (ISHLT) collects data from centers where lung transplantations are performed. In 2011, 183 centers reported 3747 lung transplantations, the highest number performed in any year to that date. The continuous increase in number consists mainly of double-lung transplantations.

The corresponding data from Sahlgrenska University Hospital (SU) in Gothenburg, Sweden over the past decade reflect the international trend, with a steady increase in the number of transplantations being performed, driven by the increasing number of recipients who receive two new lungs (Figure 1).

The rate of patient survival after lung transplantation has improved in the last decade. However, 50% of the recipients have died 5.6 years after the procedure (Figure 2). Factors that improve long-term survival include: double-lung transplantation; recipient age <50 years; and recipient being diagnosed with α-1-antitrypsin deficiency (5).

Figure 1. Lung transplantations performed at SU in the period 1990–2013



HLTx, Heart and lung transplantation; SLTx, single-lung transplantation;
DLTx, double-lung transplantation. EVLP transplantation was introduced in 2011.

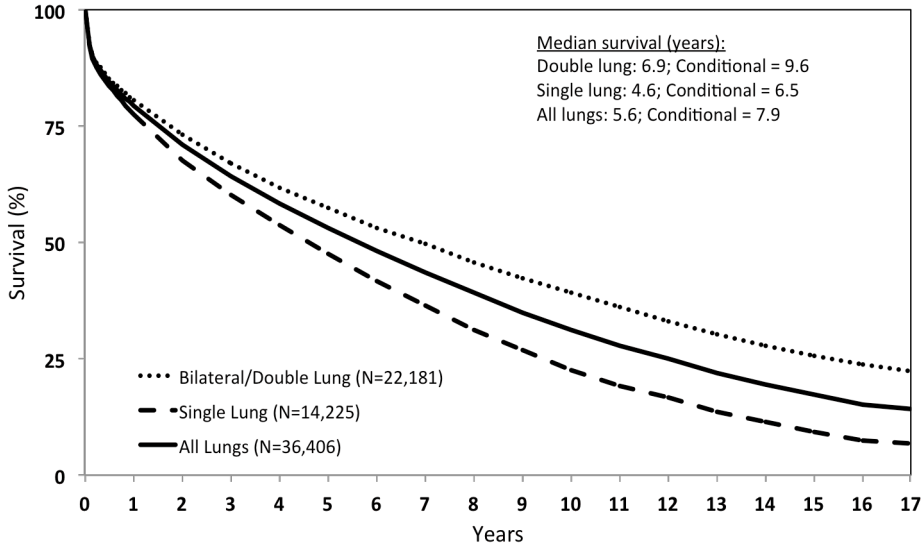
The lung transplant recipient

The shortage of donor lungs restricts the number of lung transplantations that can be performed. Patients who are wait-listed for lung transplantation are at risk of morbidity and mortality. The rate of mortality for patients on waiting lists is high. In 2011, 351 patients died while on the waiting list for a new lung in the US (6) and 50 patients in the UK (11% of listed patients) died in the same situation (7). At SU, waiting list mortality has averaged 7% in the last decade, which is a low percentage by international standards. The number of patients referred to SU for lung transplantation investigation is also increasing each year, indicating growing demand for this procedure.

Various diseases have terminal respiratory failure as the end-stage. However, only a few conditions generate the majority of the candidates for transplantation.

Chronic obstructive pulmonary disease (COPD) is generally related to tobacco smoking, although it can also be caused by α-1-antitrypsin deficiency. In COPD, gradual deterioration of the intricate alveolar structure of the lung results in impaired lung function. The symptoms are cough, shortness of breath, and frequent respiratory tract infections. If the underlying cause is α-1-antitrypsin deficiency, the onset of symptoms usually is earlier (at 30–50 years of age), as compared with the smoking-related form.

Figure 2. ISHLT data of survival after lung Transplants in adults



Kaplan-Maier survival by procedure type (1994-2011).
Reprinted with permission from the ISHLT.

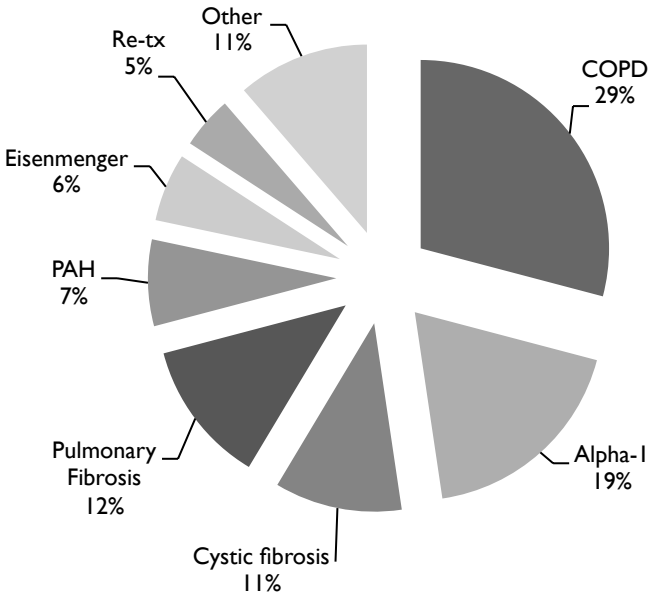
Pulmonary fibrosis (PF) is a disease that gradually impairs both the volume and the gas exchange capacity of the lungs. The fibrosis can be related to other diseases, such as sarcoidosis, scleroderma, and chronic inflammatory disorders. Environmental factors are also known to cause pulmonary fibrosis. Idiopathic pulmonary fibrosis is a disease of unknown etiology. If the patient suffers from this variant of the disease, deterioration is often rapid and the 2-year survival is 50%.

Cystic fibrosis (CF) is an autosomal recessive genetic disorder in which abnormal ion transport in the epithelium of the lung leads to thick secretions. The patient with CF suffers chronic infections of the lungs, which in turn lead to bronchiectasis and impaired lung function.

In *pulmonary artery hypertension* (PAH), the small vessels of the lungs become narrowed and lose their elastic properties. This creates a higher vascular resistance in the lungs and eventually causes right-sided heart failure. The disease can be idiopathic or associated with congenital heart disorders, viral infections, drug treatments or chronic pulmonary embolization.

The indications for lung transplantation at SU in the period 1990–2013 are displayed in Figure 3.

Figure 3. Diagnosis of lung transplant recipients at SU 1990–2013.



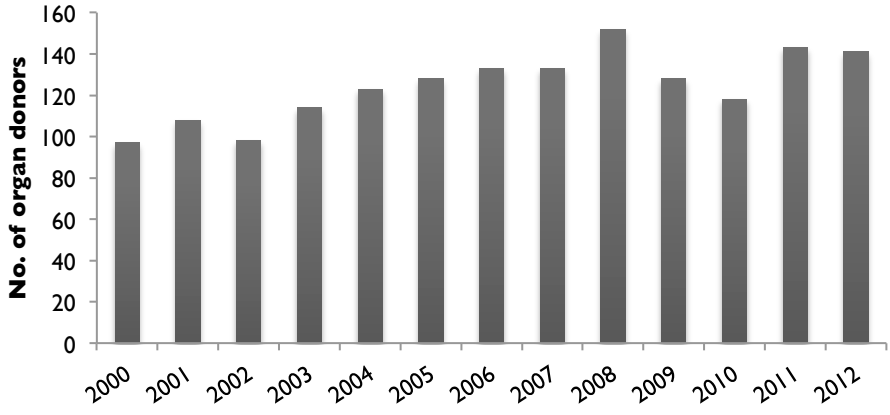
Indications for lung transplantation in patients at Sahlgrenska University Hospital (SU) in the period 1990–2013. COPD, Chronic obstructive pulmonary disease; Alpha-1, α-1-antitrypsin deficiency; PAH, pulmonary artery hypertension; Re-Tx, re-transplantation.

The lung donor

Utilization of lungs from organ donors

National data for the US reported the utilization of lungs from 21% of multi-organ donors in 2011 (6). In the UK, 1530 potential lung donors yielded 175 transplanted patients within the country (11.5% utilized) and an additional 27 lungs were exported abroad in 2011–2012 (7). After improvements in donor management and the implementation of extended donor criteria, the lungs from 30–40% of donors in Sweden have been transplanted over the last years.

Figure 4. Multi-organ donors in Sweden per year 2000–2012



Multi-organ donors in Sweden in the period 2000–2012, showing that the numbers have stabilized after increases over the first 5 years of the decade.

The number of multi-organ donors in Sweden during the past decade has increased, albeit not at the same pace as the number of lung transplantations (Figure 4). As a consequence, a higher rate of lungs from multi-organ donors has been used. To date, the success rate for lung transplantation has been unaffected by this phenomenon.

The ideal lung donor

The ideal lung donor is young, previously healthy, non-smoking, and without impairment of lung function in the period preceding donation. All of these conditions are rarely found simultaneously in a donor, and consequently, the ISHLT has defined donor criteria, which, if fulfilled, are considered optimal for ensuring good recipient outcome (8) (Table 1).

Table 1

Ideal donor criteria according to the ISHLT

Age <55 years
ABO compability
Clear chest radiograph
PaO_2> 40 kPa
Tobacco history < 20 pack-years
No chest trauma
No aspiration
No prior cardiopulmonary surgery
Absence of purulent secretions

Several publications have shown that donor lungs that do not fulfill one or several of these criteria can be transplanted without compromising long-term survival (9-11). As a matter of fact, recent data show that the majority of donor lungs that are used do not fulfill all the ISHLT criteria (9). In clinical practice, the donor circumstances are often complex, which means that individual assessment might be more advantageous than the strict following of guidelines. A major question is how much the ideal donor criteria can be extended before the results are affected. This is also where evaluation of lung function using *Ex Vivo* Lung Perfusion (EVLP) has found its way into lung transplantation. EVLP is not only a method for distinguishing extended-but-usable from unusable donor lungs, but also represents a potential tool for the treatment of donor lungs with reversible pathologies, so as to make them suitable for transplantation.

Mechanisms of impaired lung function in the donor

Lung function is often impaired in organ donors, even in patients with previously healthy lungs. Several factors may contribute to this phenomenon, such as mechanical ventilation, trauma, infections, and pulmonary edema. Severe brain injury causes elevated intra-cranial pressure (ICP), which precedes brain death. The autonomic response to elevated ICP triggers pulmonary edema. The underlying mechanism is not completely understood but most likely depends on: a) neuro-cardiac effects, whereby massive release of catecholamine induces direct myocyte injury and fluid congestion in the lungs; b) neuro-hemodynamic effects as the systemic and pulmonary pressures increase after brain injury; and c) increased

permeability of the membranes between the capillaries and the alveoli, caused by the increase in blood volume in the pulmonary vessels, and leading to vascular leakage and pulmonary edema (12).

The DCD donor

Before the classification of organ donors can be introduced, it must be emphasized that death is defined as the total and irreversible cessation of all functions of the brain.

Brain-dead, potential organ donors can then be classified based on the status of their circulation. Donors who fulfill the direct or indirect brain death criteria with circulation still on-going are classified as brain-dead donors (i.e., donation after brain death or DBD).

When brain death is preceded by circulatory arrest, the phenomenon is classified as donation after cardiac death (DCD). Non-heart-beating donation (NHBD) exists as a parallel definition of the same state. The DCD abbreviation has recently been designated as 'donation after circulatory arrest' (also DCD) due to the introduction of artificial pumps, such as in extra-corporeal membrane oxygenation (ECMO), which can sustain life without actual cardiac activity.

In a DCD convention held in Maastricht in 1995, subgroups of DCD donors were defined (13) as an attempt to classify the donors according to the circumstances in which the death had occurred (Table 2). In DCD, the donor is referred to as controlled or uncontrolled. This definition is based on the circumstances at the time of death. Uncontrolled donors (more accurately, uncontrolled death cases) are dead on arrival or have undergone unsuccessful resuscitation. Controlled donors will imminently suffer cardiac arrest or develop cardiac arrest after brain death. Recently, a fifth category of DCD has been proposed, as an unexpected cardiac arrest in a hospitalized patient may not readily fit into Group I or Group II.

Table 2 Subgroups of candidates for donation after cardiac death (DCD)

Group	Event	Classification
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Awaiting cardiac arrest	Controlled
IV	Cardiac arrest in a brain-dead donor	Controlled
V	Cardiac arrest in a hospitalized patient	Uncontrolled

Originally, only the four first categories were used. The distinction between the controlled and uncontrolled classifications have important clinical consequences and applications.

Lung transplantation from donors after cardiac death

When James Hardy performed the first human lung transplantation in 1963, the donated organs were from a DCD (1). In that era, the brain death concept had not yet been established, and all transplanted organs originated from DCD. After the introduction of the concept of brain death in transplantation in the 1970s and 1980s, organs used for transplantation were almost exclusively from DBD.

In light of the limited number of organ donors, Egan and colleagues (14) carried out a series of experiments to investigate the possibility of reintroducing lung transplantation using organs from donors after circulatory arrest; the initial studies performed on dogs, reported in 1991, demonstrated the feasibility of this concept.

In 1995, the first successful lung transplantation using a controlled (Group III) DCD donor was reported in a meeting abstract by Love et al. (15) There followed occasional case reports of DCD transplantations until the last decade, when a high number of lung transplantation centers started to use the controlled DCD procedure in their clinical practices (16-18).

Steen and co-workers in Lund, Sweden received permission from local and national ethical committees for the transplantation of DCD lungs. In 2001, this group reported the first successful lung transplantation performed with an uncontrolled DCD donor (Group I) since the introduction of the concept of brain death(19). Despite initial success, lung transplantation from uncontrolled DCD donors (Group II) is now only routinely carried at one center in Madrid, Spain (20).

Legal aspects

Swedish legislation regarding transplantation issues is based on the Transplantation Act. In 1987, the legislation for determining death was passed (*Lag om kriterier för bestämmande av människans död*; SFS 1987:269). In this law, the concept of brain death was introduced. This dramatically changed the prerequisites for organ transplantation, as patients could be declared dead although the circulation was operating and, as a consequence, organ function remained intact. The new legislation found its way into clinical practice in 1989, and provided the foundation for the still on-going increase in transplantation activity. The current law dates from 1995 and states that "All functions of the brain must be totally and irreversibly ceased" and that it is the responsibility of the physician to determine whether death has occurred by either:

- Indirect criteria - breathing and circulation have stopped and this state has persisted for a period of time that makes it possible to determine with certainty that all functions of the brain have totally and irreversibly ceased;

- Direct criteria - if breathing and circulation are maintained artificially, death shall instead be determined by an examination of the brain that with certainty shows that all functions of the brain have totally and irreversibly ceased.

Although the transplantation reported by Steen and colleagues in 2001 could be considered a prejudicial case, the legal ramifications of DCD in Sweden remain unclear. An investigation of this topic has been ordered by the Swedish government and will be executed by health authorities together with the Swedish National Board of Health and Welfare (*Socialstyrelsen*), with a start date in 2014. An advisory opinion on the legal aspects of DCD can be expected in 2–3 years. Most of the actors in the Swedish transplantation field feel that it is not reasonable to proceed with DCD before this investigation have reached a conclusion (personal communications, DCD Meeting, Stockholm, 2013).

Ex Vivo Lung Perfusion

History

Isolated perfusion of the lungs *ex vivo* has been used for more than 50 years to study lung physiology (21). Steen et al introduced *Ex Vivo* Lung Perfusion (EVLP) to clinical lung transplantation with studies of uncontrolled DCD (19, 22). In a DCD donor with previously unknown history and of unknown lung function, EVLP provides a tool for functional quality control. Steen and coworkers developed and refined the EVLP technique to use in cases of uncontrolled DCD. This approach is reflected in the design of their EVLP protocol (Table 3). Stig Steen was a physician in Toronto under the tutelage of Dr. Joel Cooper, as was Shaf Keshavjee, who also directed his research efforts towards EVLP. Unlike Dr. Steen, Dr. Keshavjee did not focus primarily on the evaluation of uncontrolled DCD lungs, but rather concentrated on developing a functional EVLP model for donor lung perfusions over longer periods of time. The development of a stable EVLP model that would work over several hours would provide the basis for interventional, *ex vivo* treatment of the sub-optimal donor lung. This approach is reflected in the protocol developed by the Toronto group under Dr. Keshavjee (23)(Table 3). In contrast the protocol of Steen et al. (22) (Table 3) is primarily designed for a shorter perfusion period and for the evaluation of initially rejected donor lungs and uncontrolled DCD lungs. The Toronto protocol is also applicable to longer EVLP and to the development of future treatments to be applied during extended lung perfusion. In 2012, a third EVLP strategy was proposed (24), in which a portable EVLP unit is used. Perfusion is instituted at the donor hospital and is maintained during transportation and up until the transplantation.

EVLP strategies

EVLP provides an alternative to the cold static preservation normally applied to organs in the period of time between removal from the donor and transplantation into the recipient. During EVLP, the lungs are not exposed to either ischemia or the depressed metabolic state that hypothermia induces. EVLP can be used for both the evaluation and reconditioning of lung function *ex vivo*. However, the warm and metabolically active state during EVLP also requires adequate perfusion, protective ventilation, and supplies of O_2 and nutrients. The graft can be damaged if these prerequisites are not met. Several groups have demonstrated the feasibility of EVLP

in animal studies and in clinical settings, and shown that different EVLP protocols can be used successfully (23, 25, 26). Therefore, the EVLP techniques implemented in the papers in this thesis should not be considered as definitive, as several strategies have been shown to produce transplantable donor lungs. Nevertheless, the optimal protocol for EVLP management should be debated.

Table 3. Comparisons of the Lund and Toronto EVLP protocols

Protocol	Pump	LA	Fluid	PAP	PA flow	Storage
Toronto	Centrifugal	Closed	Steen solution	10–15 mmHg	40% of estimated CO	On ice
Lund	Roller	Open	Steen solution + RBCs	Max 20 mmHg	Max. 70 ml/kg/donor weight	Topical Cooling

LA, Left atrium; RBCs, red blood cells; PAP, pulmonary artery pressure; PA, pulmonary artery; CO, cardiac output.

Circuit set-up

Hardware

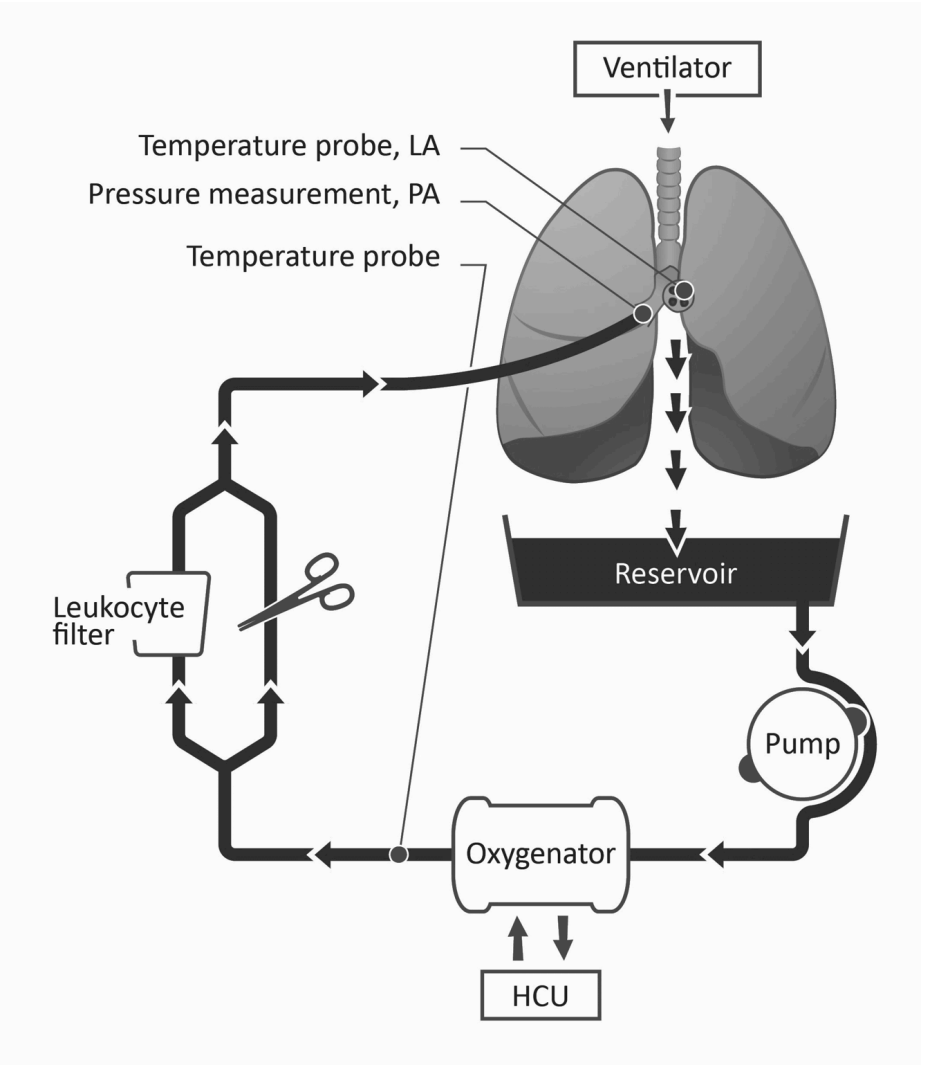
The EVLP circuit consists of the following mandatory components (Figure 5): a) a pump to create flow through the lungs, which should optimally impose a low shear stress on the perfusate and provide durability during the perfusion; b) a heater-cooler unit for warming and cooling the perfusate during the different phases of the procedure; c) an oxygenator for adding O_2 and CO_2 to the perfusate, as well as for deoxygenation during the evaluation of lung function; d) a leucocyte filter, which is part of the circuit as in a conventional heart-lung machine, although its value in the EVLP setting remains to be proven. A standard ventilator is also mandatory, although it is not considered an actual part of the EVLP circuit. Several manufacturers provide pre-assembled EVLP circuits that contain all the components listed above except the ventilator: XPS™ (XVIVO Perfusion AB, Gothenburg, Sweden); Vivoline® LS1 (Vivoline Medical, Lund, Sweden); OCS™ Lung (Transmedics, Andover, MA, USA); and Lung Assist® (Organ Assist, Groningen, The Netherlands). Many centers that perform lung perfusions choose to assemble the EVLP circuit from "off the shelf" components that are manufactured for perfusion during heart surgery.

Open or closed circuit

EVLP systems use either an open or a closed circuit. In an open system, the heart is removed before the lungs are connected to the circuit and the remnant of the left atrium is opened wide. The alternative is a closed system, which means either that the heart is kept in the EVLP circuit and a cannula collects the oxygenated blood from the left ventricle or atria, or that the heart is removed and a cannula is sewn onto the remnant of the left atrium. Advocates of the closed system suggest that this confers the benefit of controlling the left atrial pressure. In the open system, ventilation can cause intermittent collapse of the pulmonary veins, which in turn can cause congestion in the capillaries and increase the risk of damage to the alveolar-capillary membranes, leading to interstitial edema. There is evidence that both too-high and too-low pressures in the left atrium can induce pulmonary injury (27, 28).

We opted for an EVLP system that was manufactured without the possibility for closed circuit perfusion but that required an open left atrium. We learned from experience that it is important to remove as much tissue as possible from the left atrium without compromising the cuff needed for the transplantation. This is necessary to eliminate the risk of an outflow obstruction (Paper III). While we acknowledge the possibility of venous collapse, we consider risk to be low when a fountain of perfusate with a height of 2–5 cm can be observed in left atrium throughout the EVLP procedure. Venous collapse should not occur as long as this phenomenon is observed. In addition, an open atrium provides the opportunity to collect blood gases from selected pulmonary veins, thereby allowing evaluations of the functions of isolated parts of the lung. The opportunity to control left atrial pressure during EVLP is however appealing.

Figure 5. Schematic of the EVLP unit used at SU.



The blood enters the open reservoir *via* the remnant of the left atrium (LA). Samples for blood gas analyses are drawn from the pulmonary vein outflow and from a port after the oxygenator, where drugs can also be administered. PA, pulmonary artery; HCU, heater-cooler unit.

Fluids for EVLP

The perfusate used in the EVLP circuit must meet some basic criteria. The colloid osmotic, or oncotic pressure, must be equal to or higher than the oncotic pressure in the blood. An oncotic pressure of about 25 mmHg normally exists in the capillaries. The hydrostatic pressure in the arterial end of the capillaries is normally about 30 mmHg, and the hydrostatic pressure in the venous end of the capillaries is normally about 10-15 mmHg. This pressure gradient causes water to leave at the arterial end and return at the venous end of the capillaries.

The commercially available solution that is currently used almost exclusively for EVLP in both laboratory and clinical settings is Steen Solution (XVIVO Perfusion AB). This solution has a composition similar to that of the extracellular fluid. The inclusion of human albumin and dextran 40 increases the oncotic pressure and inhibits coagulation. As the name implies, Stig Steen and colleagues developed this solution. This fluid has a high oncotic pressure (25–30 mmHg), which the manufacturer described as optimal for mobilization of the interstitial edema often found in donor lungs.

The hyper-oncotic property of Steen solution is however not constant if the solution is used for perfusion of an edematous lung. As the edema from the interstitial space of the lung is gradually mobilized into the perfusate, the oncotic pressure of the latter will gradually reach equilibrium with the interstitial space of the lung. Thus, the ability to mobilize further fluid will be diminished. Strategies to overcome this problem have been suggested. Hourly exchange of part of the perfusate has been proposed to counteract this phenomenon (23). However, the effectiveness of this method remains to be proven. In 2013, we presented in a case report an alternative approach in which a hemo-concentrator was included in the EVLP circuit. Using this set-up, the perfusate could be dialyzed of a chosen amount of fluid over time which meant that the oncotic pressure could be maintained at super-normal levels (29).

Red cells in the perfusate

The group of Steen has advocated the addition of red blood cells (RBC) to the perfusate, whereas the Toronto group has proposed that the added RBCs limit EVLP to time periods shorter than 120 minutes (30). Support for the latter theory is cited by referring to a paper in which four uncontrolled DCD porcine donor lungs were subjected to 6 hours of EVLP; in that study, the perfusion was restricted to a low flow rate due to high pulmonary vascular resistance (PVR) (31). During prolonged perfusion, a roller pump will cause trauma to the RBCs. The hemolysis will release hemoglobin, which can act as a scavenger of nitric oxide and thereby increase the PVR (32). If a longer EVLP procedure is planned, the combination of a roller pump and an RBC-containing perfusate may not be optimal.

To date, no study has compared EVLP that uses an acellular perfusate with EVLP that uses a cell-containing perfusate in a large animal model. In a small series Yeung et al. (33) perfused lungs during EVLP, first with an acellular perfusate and subsequently with a perfusate that contained RBCs to a hematocrit of 10%. During perfusion, the left main bronchus was clamped, thereby creating a large ventilation/perfusion mismatch. During acellular perfusion, no significant difference was noted for the PaO_2 after the bronchial clamping. With an RBC-containing perfusate, the PaO_2 dropped significantly (33). Although this finding did not prompt the authors of this trial to alter their strategy to use an acellular perfusate, it indicates that in a situation where there is a large shunt a perfusate that contains RBCs is a more sensitive instrument for the detection of impaired oxygenation capacity.

About 2.9 ml of O_2 is dissolved in every 1000 ml of water at a PaO_2 of 12 kPa. One gram of hemoglobin (Hb) binds 1.38 ml of O_2 when fully saturated (34). In an EVLP circuit with 2.5 L of perfusate with a Hb concentration of 30 g/L (hct of 10%), a total of 7.5 ml of O_2 is transported dissolved in the plasma, and a corresponding volume of 103 ml is bound to Hb when saturated to 100%. This implies that the O_2 content of a solution with a Hb concentration of 30 g/L is more than 13-fold higher than that of a corresponding acellular solution. To attain a certain level of PaO_2, much higher demands are made of the oxygenation capacity of the lung (i.e., O_2 transport from alveoli to capillary) in a perfusate that contains RBCs, as compared with an acellular perfusate. Consequently, adding RBCs to the EVLP perfusate provides a more stringent test of lung function.

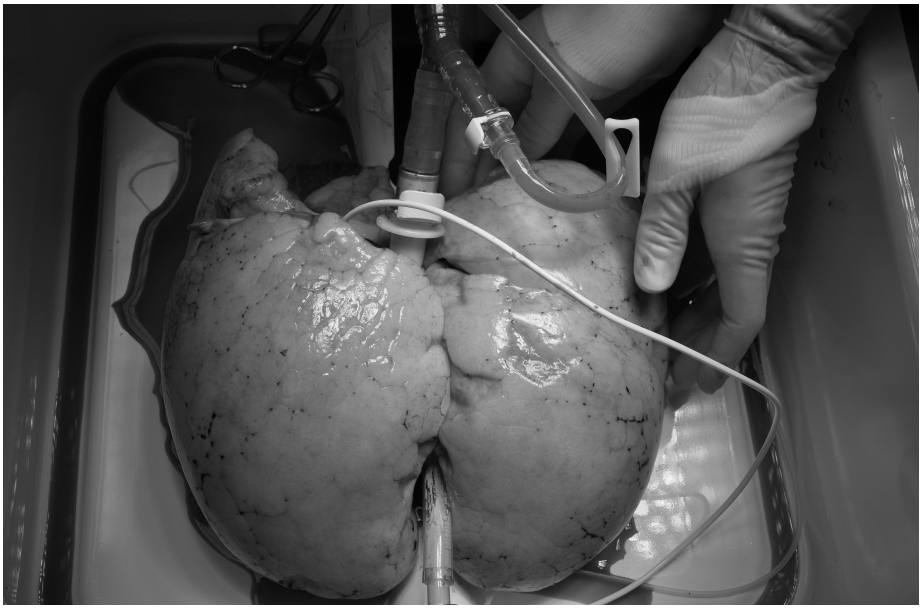
In the animal studies included in this thesis (Papers I and II), a separate blood group-matched animal was exsanguinated and used as a blood donor. The blood was washed in a cell-saver before being added to the EVLP priming solution. In the clinical studies, bank blood, which was cross-tested and matched to the recipients, were used for priming the EVLP circuit. Opponents of the blood-containing perfusate regime have experienced logistical difficulties in ordering bank blood when there is *de facto* no physical recipient. This has not been a problem in our clinic.

Even if the ventilator and hemodynamic variables are adequate for determinations of donor lung quality, we cannot find any reason to exclude the well-established blood gas analysis for assessments of the blood oxygenation capacity of the lungs during EVLP. Therefore, we continue to add RBCs to the perfusate to a hematocrit of 10%–15%.

Pressure and flow rates during EVLP

A similar EVLP protocol was applied in the animal and clinical studies included in this thesis. EVLP blood flow is regulated by a pre-set pressure limit, as well as by a pre-set limited flow rate. During EVLP, the intrinsic pulmonary vascular resistance (PVR) often regulates the flow. This is especially true during the re-warming phase of EVLP when the flow is always limited by the high PVR in the cold and rigid lung. Once again, the approaches taken by the Toronto and Lund groups are different (Table 3). The Toronto group relies on an EVLP technique with low PA flow (40% of the estimated cardiac output), low PA pressure (10–15 mmHg), and advocates a careful perfusion strategy with initially very low PA pressures. To

Picture 1



Human donor lung connected to EVLP. Tracheal tube at 6 o'clock. PA inflow at 12 o'clock. Temperature probe diagonal across the lungs. Shunt at 1 a'clock is closed.

facilitate perfusion over longer time periods, the maximum pressure is kept low throughout the entire EVLP procedure. This regime has been shown to be unharmful to porcine lungs during 12 hours of perfusion (23).

The theoretical disadvantage with the low-flow regime is that a low pressure level could be insufficient for perfusion of the non-dependent parts of the lung, e.g., West zone 1. In an uncontrolled DCD situation with a lung that has impaired peripheral circulation, the use of a to careful perfusion regime may result in that only a part of the lungs are perfused. In combination with an acellular perfusate,

which entails unreliable blood gas recording, a 'bad' lung could be considered suitable for transplantation.

In contrast, the Lund protocol uses a pre-set pressure limit of 20 mmHg from the start to the end of the perfusion. Thus, the flow is more dependent upon the PVR. The latter strategy was applied in the animal studies (Papers I and II), mainly because the experiments were conducted in close co-operation with Dr. Steen. At the start of our clinical EVLP program, we applied an approach that was slightly modified based on our experimental experiences. As our own experience increased, we gradually adjusted our regimen. Lower pressure limits (10–15 mmHg) were used during the reconditioning phase of EVLP, to maintain a protective regimen for the cold lung. Soon after the introduction of clinical EVLP, we recognized the importance of lowering PA flow during lung recruitment maneuvers. As the peak end expiratory pressure (PEEP) is increased, so is the pressure on the venous side of the lungs. The outflow obstruction created will push fluid from the vessels into the interstitial space and subsequently, the alveoli, thereby creating a pulmonary edema. This was later demonstrated in a study conducted by Lindstedt et al. (35). In contrast to the EVLP protocol applied by the Toronto group, we continue to evaluate lungs at full PA flow and pressure (20 mmHg). This is done with the ambition that the testing of lung function during EVLP should, as far as possible, simulate the conditions under which the lungs will subsequently be required to function.

Ventilation during EVLP

A recent Cochrane review showed the superiority of a so called protective ventilation strategy with low tidal volumes over a strategy with higher (physiological) tidal volumes in acute respiratory distress syndrome (ARDS)(36). The ventilation settings used by many centers during clinical EVLP is similar to what is applied for patients with ARDS (23, 24, 37). We also recognise this strategy as optimal during EVLP although we, in the early clinical EVLP experience, ventilated the lung with larger volumes (Paper III).

Phases of the EVLP procedure

Irrespective of the EVLP protocol used, the procedure has distinct phases. The EVLP procedure, as it is performed at SU, is described in the following sections:

Preparation

When the donor lungs arrive at the operating theater of the receiving center they must be prepared for the EVLP procedure. Superfluous tissue is trimmed off and the lungs are weighed. Cannulas are secured in the trachea and the pulmonary artery. If the donated heart is favored and the pulmonary artery is cut at or after the

bifurcation, the artery must be elongated with a graft. This was performed for 9/22 EVLP cases in Paper IV. The graft may consist of a piece of the donor aorta or a synthetic graft. The lungs are connected to the EVLP unit, and a temperature probe is sutured to the remnant of the left atria to record the temperature of the blood leaving the lungs. In the Vivoline EVLP disposable a thin catheter is provided. It is constructed for placement in the left atria, to allow blood sampling throughout the procedure. In our initial employment of the EVLP system, we used this accessory. During one of the first clinical perfusions, the catheter slipped into the vein from the right upper lobe without being noticed. This meant that the blood gases were drawn selectively from one lobe. In this pair of lungs, this particular lobe had the worst function. When blood gases were instead taken from the mixed blood in the left atrium, a more complex and accurate evaluation of lung function could be made. Thereafter, we did not use the provided catheter. When selective blood gases from a particular part of the lung are required, blood is drawn from a syringe placed in the corresponding pulmonary vein.

Warming and reconditioning

After the lungs are connected to the EVLP unit, they are gradually rewarmed to body temperature. Bronchoscopy is performed for airway control and cleaning. At 32°C, ventilation is initiated with small tidal volumes and at low pressure. Since the warming of the perfusate takes place with a pre-set difference in temperature of 8°C between the in- and out-flowing blood, the duration of the warming is dependent upon flow and the size of the lungs. The median times to reach 32°C and 36°C in our clinical series were 30 and 47 minutes, respectively.

The warming phase is also referred to as the reconditioning phase, as it is the time during which there is mobilization of airway debris and recruitment maneuvers of atelectasis.

Evaluation

Once a temperature of 36°C is reached, the evaluation phase is started. At this point, the O_2 supplied from the EVLP unit is discontinued. In this phase, the oxygenator is only used for deoxygenation and the provision of CO_2. Oxygenation and removal of CO_2 from the perfusate are now dependent upon lung function, which can be evaluated by analyzing the blood gases. The lung compliance, dead space fraction, respiratory pressures and PVR are evaluated repeatedly during this phase. Some EVLP centers perform multiple evaluations over several hours of EVLP. Our strategy has been to perform evaluations once the lung is warm and well-recruited. If lung function is deemed to be sufficient for transplantation, there is no reason to prolong the EVLP, and instead, we proceed to transplant the lungs. We argue that the recipient is a better environment for the lung than the EVLP unit.

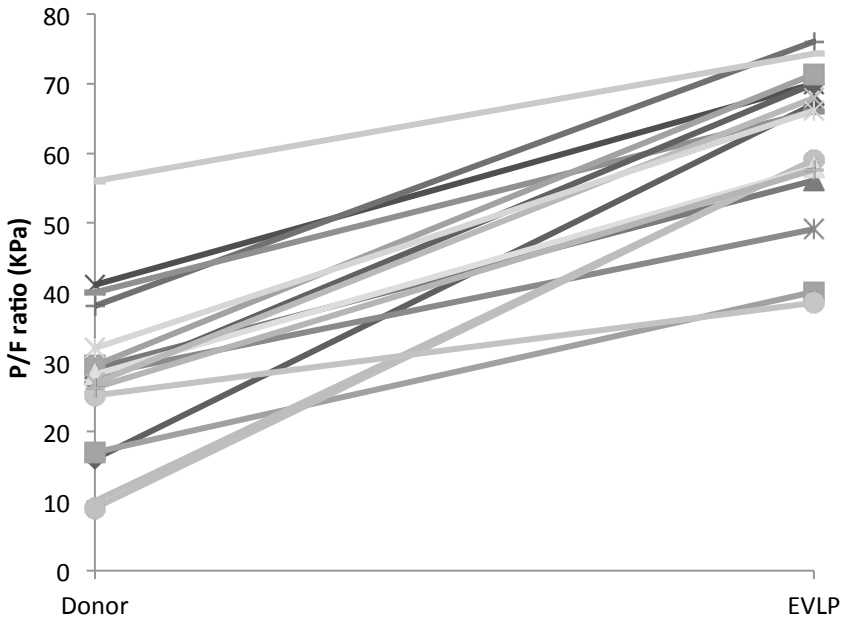
Cooling and storage

If the lungs are deemed transplantable, the oxygenator is once again used as an O_2 provider. The lungs are cooled to 12°C. Ventilation is terminated at 32°C. For the first two clinical EVLP cases, we stored the lungs in Perfadex on ice. This is also the preferred method in the Toronto protocol (23). Starting at the third clinical EVLP, we now keep the lungs in the EVLP circuit. The lungs are not perfused but instead embedded in a compress in an inflated state. The cold perfusate is flushed over the lungs at 8°C. In the case of double-lung transplantation, one lung is maintained in the EVLP circuit while the other is transplanted.

Improving lung function during EVLP

In concordance with the results shown in Papers III and IV (Figure 6), several studies have reported improved blood gas recordings when pre- and peri-EVLP blood gases are compared (38).

Figure 6. Data from the EVLP program at SU for the period 2011–2013



The P/F ratio was recorded in 17 of our donors when a decision was made to reject for transplantation and instead proceed to EVLP. On the right-hand side of the graph are the P/F ratios registered during EVLP. Each line represents one evaluated pair of donor lungs. All evaluated lungs showed improvements in oxygenation capacity.

The mechanism for this improvement is primarily dependent upon:

- Bronchial secretions that effectively can be cleared from the lung before ventilation is started.
- Controlled recruitment of atelectasis. The evaluation of the lungs during the *ex vivo* phase provides optimal conditions, as the ventilator pressures can be gradually increased under visual inspection of the lung.

Several potential advantages and treatment suggestions have also been proposed that *may* contribute to improved lung function during EVLP. The use of an EVLP perfusate with an oncotic pressure higher than the pressure in the interstitial space could theoretically mobilize edema from the donor lung (29). Results from paper III demonstrates that lungs with a relatively low weight before EVLP gained weight during the procedure. When the haematocrit was analysed during the course of the EVLP it increased in all cases in Paper III. This could be considered as signs of a fluid shift from the perfusat to the lung and contradict the theory of an absorbing effect of the perfusat. The optimal oncotic pressure of the perfusate remains to be elucidated. The addition of an antibiotic to the perfusate has been shown to decrease the bacterial load in the donor lung after EVLP (39). Thus, infections in the recipient may be reduced. Micro-thrombi may be washed out during the EVLP. Donor leukocytes could be washed out and trapped in the leukocyte filter that is included in the EVLP circuit. The use of an adsorbent membrane to decrease the level of passenger cytokines has also been investigated (40). Several other treatment regimens have been suggested, such as thrombolytic treatment during EVLP (41), and the inhalation of surfactant after suspected aspiration of gastric contents (42). The use of gene therapy has also shown promising results, although it has not been applied to clinical lung transplantation (43).

Evaluation of lung function during EVLP

Arterial blood gases

Oxygenation capacity is traditionally considered to be the most important parameter for the evaluation of donor lung function. During EVLP, blood gases are drawn from the oxygenated blood in the left atrium. The gas exchange in the evaluated lung at a certain fraction of inspired oxygen is dependent upon the following factors: a) the ventilation of the alveoli; b) the diffusion capacity of the blood-gas barrier; c) the amount of shunted blood; d) the ventilation/perfusion relationship.

The prognostic value of the oxygenation capacity of the lung during EVLP has been questioned by the Toronto group (33). Gradually decreasing dynamic compliance over time is instead suggested as the best indicator of inferior lung function (33).

The use of an acellular perfusate might explain why this model fails to prove the advantage of blood gas analysis. The assumption made by the Toronto group is however supported by a review of the UNOS registry, in which the DBD P/F ratio did not affect recipient survival (11). That publication provides some indirect evidence that the P/F ratio during EVLP has a limited predictive value regarding lung function in the recipient. However, evaluation of lung function in this paper (11) is not conducted under EVLP circumstances and in donors with lung function that is already deemed to be sufficient for lung transplantation. Paper IV supports the use of blood gases as an important prognostic parameter for recipient outcome, since the recipients of lungs with the lowest P/F ratios during EVLP seemed to have the most cumbersome post-operative course of events.

PVR

In both animal and human EVLP, irrespective of the EVLP protocol employed, the PVR is higher than what would be expected for normal *in vivo* physiology (26, 38) (Table 4). This phenomenon might be explained in part by the difference between the physiologic flow *in vivo* and the artificial flow generated by the pump in the EVLP unit. The EVLP perfusate has a lower viscosity than blood, and this would decrease rather than increase the PVR. It has been debated whether perfusion for more than 2 hours is possible with a high flow rate and a perfusate that contains RBCs (30). In our clinical series (Papers III and IV), the lungs underwent EVLP for up to 5 hours without any marked increase in PVR over time. At the end of EVLP, when the cooling phase is instituted and the lung becomes cold and less compliant, it is expected that the PVR tends to increase, although a high or increasing PVR during the normo-thermic phase of EVLP might be an indicator of increased tissue edema.

Compliance

The compliance of the lung reflects the change in lung volume for an applied pressure (Table 4). Static compliance is calculated during an inspiratory hold maneuver (static condition). Compliance is calculated as the inspired tidal volume is divided by the insufflation pressure at the end of inspiration (plateau pressure) minus the positive end-expiratory pressure. Compliance, and especially changes in compliance over time, has been suggested as important parameters for evaluating donor lung function during EVLP (33). However, the predictive value of high donor compliance with regard to good lung function in the recipient has not been studied in the EVLP situation or, as far as we know, in the conventional DBD situation. The existence of a relationship seems logical, as a lung that has a barrier leak will get stiffer during EVLP and thereby decrease its compliance.

Table 4. PVR compliance levels reported in various studies and in the papers from this thesis.

	Human				
	In vivo†	Cypel (38)	Ingemansson (26)	Aigner (37)	Paper V
PVR	20–130	200–300	300–500	150–300	250–600
Compliance	60-80	80	n/a	75–100	60–100
	Pig				
	In vivo†	Steen(22)	Sanchez (44)	Paper I	Paper II
PVR	150	300–400	600–900	550–750	650–750
Compliance	30–35	n/a	30††	40††	40††

PVR, Pulmonary vascular resistance (dyn*sec*cm^{-5}). Compliance (ml/cm H_2O). †Normal physiology under mechanical ventilation; ††static compliance; n/a, no available data.

Lactate production

Despite being the organ with the highest O_2 concentration in the body, about 40% of the glucose that is metabolized in the lung becomes lactate (45). As the EVLP circuit is closed and the lungs ability to consume lactate is limited, the lactate level in the lung increases during the EVLP (Paper III). The level of lactate during EVLP has however been found to be unrelated to the function of the perfused lung (46).

Glucose consumption

In Paper III, the glucose levels in the perfusate are presented. The longer the perfusion persists, the lower the glucose concentration becomes. A high rate of glucose consumption during EVLP has been suggested as a marker of graft quality (47). In a group of animals with high glucose consumption, pulmonary edema was more pronounced than in the controls. However, gas exchange capacity and hemodynamic parameters showed no association with the rate of glucose consumption (47). As the level of insulin (48), lactate (49), and lung tissue ischemia (50) all influence the consumption of glucose, this parameter does not provide a solid basis for the evaluation of lung function during EVLP. The idea of replacing consumed glucose during a prolonged EVLP is appealing but has not been studied.

Lung Weight

Edema of the donor lung is a frequent cause of impaired function. The presence and amount of edema is often determined by chest x-ray or by measuring the gas exchange capacity (blood gases). In the laboratory setting (Papers I and II), a resected part of the lung can be weighed and then dried in an oven until no further weight loss is recorded. The wet to dry weight ratio is then a measure of tissue

edema. As described in Paper III, we weighed the donor lungs before and after EVLP, to quantify the pulmonary edema. As far as we know, the impact of the donor whole lung weight on lung recipient outcome has not been investigated in clinical lung transplantation. It has been shown that increased extravascular lung water content (edema) is a predictor of intensive care mortality in non-transplanted patients with acute lung injury (51). As demonstrated in Paper III, human donor lungs that have a pre-EVLP weight of <1000 g seem to increase in weight during EVLP, whereas lungs with a pre-EVLP weight of >1000 g decrease in weight.

Preservation of the lung in cases of uncontrolled DCD

General considerations

In uncontrolled DCD, the donor is either dead upon arrival at the hospital or has undergone unsuccessful resuscitation in the emergency room (Table 2). Steen et al. (19) introduced uncontrolled DCD after failed resuscitation in 2001, by transplanting a lung after evaluating its function during EVLP. Subsequently, Antonio and colleagues in Madrid implemented uncontrolled DCD in clinical practice without evaluation *ex vivo* prior to transplantation (20). The lung function was instead evaluated with blood gas taken from a single shot of Perfadex mixed with blood administered through the ventilated lung. In the latter publications, lung transplantation after uncontrolled DCD was associated with a higher incidence of graft dysfunction than would be expected for donation from brain-dead donors (52). This may have been due to the prolonged period of warm ischemia used, the inflicted traumatic lung injury in conjunction with resuscitation or the fact that the donor history and medical status were partially unknown at the time of transplantation. Good lung preservation in combination with functional evaluation during EVLP is therefore of the utmost importance in uncontrolled DCD lung transplantation (53). Legislation in Sweden prohibits intervention for organ preservation purposes before the declaration of death. In addition, interventions after death should be kept at a minimum to facilitate acceptance from next-of-kin for the uncontrolled DCD procedure. A well-educated staff and optimal logistics are essential to take care of the next-of-kin and to limit the warm ischemic time (19, 20).

Lung circulation physiology in uncontrolled DCD

When ventricular fibrillation occurs in a patient, the residual arterial pressure gradually transfers the circulatory blood volume to the venous system, the arterial pressure gradually diminishes, and the right atrial pressure increases. Eventually, a large volume of blood is transferred into the pulmonary vessels until the aortic valve, beyond which the pressure is higher, stops it. After about 5 minutes, a mean systemic filling pressure is reached, as the blood pressure is about 10 mmHg on both the arterial and venous side. Total circulatory arrest has occurred at this stage.

The right atrium and ventricle are now highly distended. If chest compressions are started, a high cardiac output is achieved during the first 2–3 minutes or as long as there is blood in the thorax (54, 55). After this period, cardiac output will depend on the venous return. However, owing to the high central venous pressure induced by the chest compressions, it is difficult to attain a cardiac output higher than 15% of what is normally achieved with manual compressions or higher than 30% of what is normally achieved with mechanical compressions. This means that the uncontrolled DCD donors, as well as the animals described in Papers I and II have been exposed to cardio-pulmonary resuscitation (CPR) for nearly 20 minutes, which creates extensive pulmonary stasis. This phenomenon also means that the lungs are filled with blood, which contains an O_2 reserve that favors the pneumocytes during the warm ischemic period in the DCD situation. Despite the inevitable trauma inflicted upon the lungs during CPR, this does not necessarily affect negatively the outcome for recipients of such a donor lung. This has been studied for the recipients of lungs from DBD donors who were or were not treated with CPR (56).

Heparin

Lung transplantations from controlled DCDs (Maastricht III) are performed routinely in several institutions around the world (16, 57-59). The results obtained are comparable to those seen with transplantation of lungs from DBDs (60). Some centers administer intravenous heparin to the potential donor before the awaited cardiac arrest (16, 58), while others do not (17, 57). In an uncontrolled DCD pig model, Steen et al. (22) introduced a preservation method for the potential lung graft that included post-mortem i.v administration of heparin, followed by a series of chest compressions to promote circulation of the heparin to the lungs.

Inokawa et al. (61) have reported that heparin improves graft function in the uncontrolled DCD setting. In their canine lung transplantation model, a high dosage of heparin (1000 IU/kg) was administered after cardiac death and this was followed by 20 chest compressions. The lungs were flushed in an antegrade fashion, explanted, and the left lung was transplanted after 2 hours of warm ischemia. No intrapleural cooling was instituted. Recipients of heparinized lungs had better lung function than recipients of non-heparinized lungs. Sanchez et al. (44) investigated the effect of pre-arrest heparin administration in a pig model in which cardiac arrest was induced with an electric shock. After 1 hour of warm ischemia, the lungs were subjected to a retrograde flush, explanted, and stored on ice for 6 hours. Evaluation was carried out during EVLP according to the Toronto protocol. The heparinized lungs showed significantly better hemodynamic function, lower wet-dry ratio, and better oxygenation capacity than non-heparinized controls. Rega et al. (62) demonstrated excellent lung function from non-heparinized, controlled DCD lungs

after 90 minutes of warm ischemia and 24 hours of cold storage. However, the protocol did not include a resuscitation step, which would be potentially harmful to the donor lungs. The same group has in two different studies stated the superiority of *in situ* topical cooling and confirmed that 1 hour of warm ischemia does not affect negatively pulmonary graft function (63, 64). In the latter study they also showed that the graft performance of DCDs without heparin administration was equivalent to that observed in a DBD control group. Once again, the lungs were not subjected to resuscitation.

In Paper I, using a pig model, which simulates the clinical situation in the uncontrolled DCD setting, we investigated whether or not heparin administered after death affects donor lung function (Picture 2). The timeline for the experiments is outlined in Table 5. EVLP was performed with the Vivoline® system and according to the protocol of the Lund Group (for details, see EVLP chapter). Lung function was evaluated with blood gases for different O_2 levels, PVR, wet/dry weight ratios, macroscopic appearance, and histology.

Table 5. Timeline for the uncontrolled DCD experiments described in Paper I.

Time (min)	
0	Ventricular fibrillation
7	CPR with mechanical ventilation and manual ventilation
27	Hands-off period. Randomization to heparin or placebo treatment
37	Declaration of death. Intravenous administration of heparin to treatment group. Two minutes of chest compressions and ventilation in the treatment group but not in the control group
39	Activated clotting time measurement
100	Placement of chest tubes and the start of intrapleural cooling
220	Harvesting of lungs following antegrade and retrograde flushing
250	Lungs connected to the EVLP. Start of the evaluation

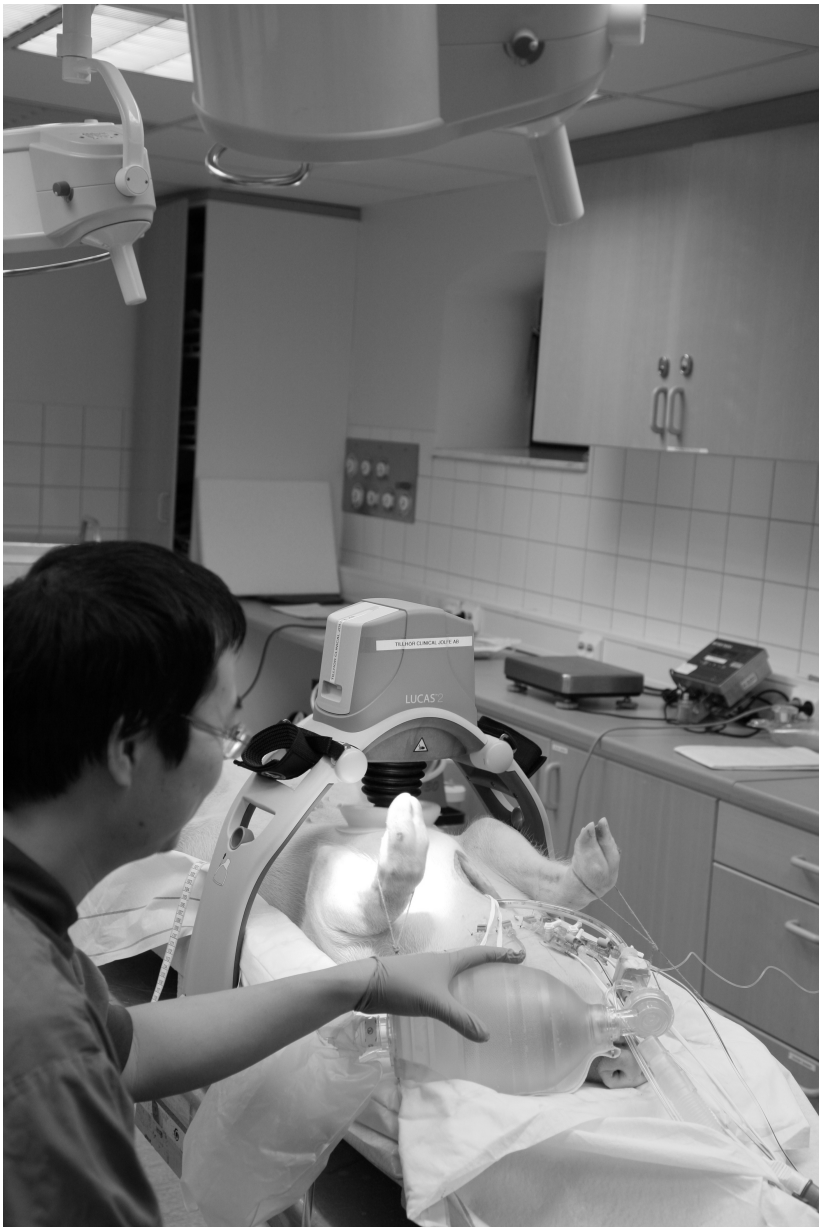
CPR, cardio-pulmonary resuscitation; EVLP, Ex Vivo Lung Perfusion.

The significantly longer activated clotting time in the heparin group confirmed that the administration of heparin was adequate. During EVLP we found no significant differences between the heparin-treated and non-heparin-treated groups (Table 6) with respect to PaO_2 or PVR at any investigated FiO_2 level (1.0, 0.5, and 0.21). For the wet/dry ratio, there was no significant difference between the groups. In the histologic examination of the lung after EVLP, findings consistent with fat embolization were obtained for a few samples. Fat embolization is often observed in conjunction with severe trauma, and likely originates from fractures in large bones. In this setting, the embolization originated from sternal and costal fractures caused by the CPR.

Table 6. Lung function parameters during EVLP.

	Heparin Treatment		No Heparin Treatment		*p*-value
	Median	Range	Median	Range	
PaO_2					
FiO_2 - 0.5	22.3	14.8–28.0	23.3	8.2–33.5	0.59
FiO_2– 1.0	63.7	57.3–73.1	62.8	51.3–70.9	0.82
FiO_2– 0.21	12.7	10.3–16.3	12.5	9.5–14.5	0.82
$PaCO_2$					
FiO_2– 0.5	4.2	3.8 – 5.4	4.8	3.7–5.8	0.39
FiO_2– 1.0	4.1	4.0–5.5	4.6	3.7–5.4	0.49
FiO_2– 0.21	4.1	3.6–4.8	4.7	3.5 – 5.3	0.31
PVR					
FiO_2–0.5	564	407–1365	657	410–1218	0.82
FiO_2– 1.0	525	402–1007	552	426–1044	0.70
FiO_2– 0.21	758	400–1156	747	443–1340	0.21
Wet/Dry ratio	5.9	5.1–6.5	5.8	5.3–7.3	0.70

No significant differences were noted between the groups for:
PaO_2 (kPa); $PaCO_2$ (kPa); PVR (dyne*sec/cm5) or wet/dry ratio.



Picture 2

Experimental set-up for the cardiopulmonary resuscitation model used in Papers I and II, involving mechanical compressions and manual ventilation.

Two of the animals in the heparin group developed intrapulmonary hematomas during EVLP. This is probably not only related to the heparin but also to the chest compressions that were applied after the administration of heparin. Chest compressions after cardiac death may cause lung contusions and may also transport embolic material into the pulmonary circulation. In our experimental setting, the formation of thrombi occurred in the main pulmonary artery when heparin was not administered, although larger thrombi were easily removed manually from the pulmonary artery and the left atrium. Smaller clots, which were occasionally observed, seem to be eliminated during the pulmonary flushing. Moreover, the lung has an excellent thrombolytic capacity. Van De Wauwer et al. (65) have previously demonstrated a positive impact of retrograde flushing on non-heparinized DCD, and more recently that retrograde flushing is more protective than post-mortem heparinization (66). Retrograde flushing together with a shorter warm ischemic time may explain why there was no difference between the groups in our study in contrast to the findings of Inokawa et al. (61) and Sanchez et al. (44). It has been suggested that heparin has positive effects other than preventing thrombosis on the pulmonary graft. Brown et al. (67) have demonstrated that heparin inhibits neutrophil activation, thereby protecting the bronchial epithelial cells from neutrophil-induced injury. This lends support to the idea of administering heparin to the donor lung, but not necessarily to the donor, when it can be administered during EVLP.

In Paper I, lung function levels, as evaluated during EVLP, ranged from excellent to abysmal in both the Heparin group and the Control group. The DCD model, which involves chest compressions, mechanical ventilation, and a warm ischemic period, results in destructive trauma to the donor lungs. Therefore, in the uncontrolled DCD setting, there is a need to evaluate the donor lungs by EVLP before transplantation. In summary, we found that the post-mortem administration of heparin conferred no obvious benefit on the donor lungs in the uncontrolled DCD situation. The omission of heparin in this situation simplifies donor management and allows time for the next-of-kin to spend undisturbed time with the deceased relative.

Lung preservation *in situ*

One hour of warm ischemia performed *in situ* on the lungs has been proven safe from the lung function perspective in DCD, whereas a longer period of warm ischemia results in organ damage (63, 68, 69). One hour should provide sufficient time to determine from the next-of-kin or from registries the wishes of the deceased regarding donation, and also allows time to prepare for the organ preservation procedures.

Various techniques have been proposed for the preservation of the pulmonary graft inside the donor. Ventilation of the donor lungs post-mortem has been demonstrated to improve and preserve graft function (70, 71). Cold storage of donor lungs in a fluid at 8°C has been shown to provide excellent lung preservation for 12 hours when pigs transplanted with such lungs were monitored for 24 hours. This finding raised the possibility of preserving DCD lungs *in situ* by infusion of a cold fluid into the pleural cavities (72). Rega et al. (63) compared ventilation with *in situ* cooling of donor lungs and found the that cooling was the superior method. Described techniques for intra-pleural cooling include intermittent shifting of fluids (53, 63) and the use of pumps to circulate the cold fluid (73). These two methods will likely require specialized expertise and may not allow for the next-of-kin to stay with the deceased. Using a syngeneic DCD rat model, Wierup et al. (74, 75) simplified the *in situ* cooling procedure by introducing a single cold (4°C) intrapleural infusion, which preserved the donor lung at 25°C for 2 hours *in situ*; one lung was then transplanted and lung function was evaluated after 5 weeks, with excellent outcomes in terms of function and bronchial healing.

In Paper II, we investigated whether the results obtained by Wierup et al. (74, 75) could be replicated in a large animal setting, with the idea that if this could be proven, then simplified cooling during uncontrolled DCD would be brought closer to clinical reality. An uncontrolled DCD model (Table 7), similar to the one described in Paper I but now without heparin, was established. Twelve pigs were randomized to intrapleural lung cooling using either a standard method with two bilateral chest tubes and intermittent pleural fluid exchanges or a simplified, less-invasive method with a single bilateral chest tube and filling of the pleural space without fluid exchange. Lungs were explanted, and graft function was assessed during EVLP, using histologic examination and analyses of the levels of myeloperoxidase in the bronchoalveolar lavage fluids.

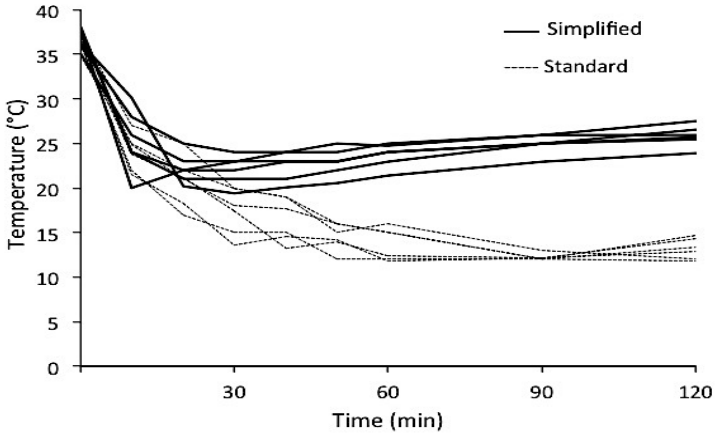
Table 7. Timeline for the uncontrolled DCD experiments described in Paper II.

Time-point (min)	
0	Ventricular fibrillation
7	CPR with mechanical ventilation and manual ventilation
27	Hands-off period.
37	Declaration of death. Randomization to cooling procedure
100	Placement of chest tubes and initiation of cooling
220	Harvesting of lungs after antegrade and retrograde flushing
250	Lungs connected to EVLP and start of the evaluation procedure

CPR, Cardiopulmonary resuscitation; EVLP, Ex-vivo Lung Perfusion.

The simplified intrapleural cooling method caused a rapid decrease in intrabronchial temperature to about 22°C. During the next 2 hours, the temperature gradually increased (Figure 7). In donor organ procurement, cooling is essential to slow the degradation of the tissues. However, it is unclear to what extent the lungs need to be cooled in order to preserve adequate function, and the optimal preservation temperature has not been established. It is generally assumed that cooling to temperatures in the range of 4°–10°C is needed to block tissue degradation (63, 68, 76, 77). Nevertheless, Steen et al. (19) conducted a successful transplantation of a patient with lungs from an uncontrolled DCD in which the lungs had been cooled to 18°C for 2 hours. When organ function was evaluated during EVLP (Paper II), no difference was found between the lungs preserved at 15°C and those preserved at 25°C (Table 8). The wet/dry ratio was not different between the groups, and the histologic investigation revealed moderate and severe signs of lung injury in just a few samples, which were evenly distributed between the standard and simplified groups. The levels of myeloperoxidase, which is an indicator of acute lung injury, in the bronchoalveolar lavage fluids were similar for the two groups. The reported P/F ratios of pig lungs during EVLP are comparable to the values that we obtained in Paper II, and within the range of 40–60 kPa demonstrated in similar studies (22, 73, 78).

Figure 7. Intrabronchial temperatures during intrapleural cooling *in-situ*.



Tracheal temperatures measured at 10-minute intervals during the first hour and after 90 minutes and 120 minutes of intrapleural cooling. Each line represents an individual. Lungs cooled using the standard method (four chest drains and intermittent fluid exchange) have lower temperatures at 60 minutes and 120 minutes than lungs cooled using the simplified method (two chest drains and no fluid exchange) (p=0.004).

While the PVR did not differ between the groups, it was high compared with the normal, physiologic values *in vivo*. That the PVR is higher than expected during EVLP has also been noted by others (42, 53, 73).

A correlation analysis was performed but it failed to demonstrate any connection between the intra-bronchial temperature during *in situ* preservation and lung function evaluated during EVLP.

The results presented in Paper II indicate that a simplified technique for intrapleural cooling can be used, and that moderate cooling to 25°C is sufficient during the first 2 hours of cold ischemia. Additional intrapleural flushes should of course be performed if prolonged cold preservation is required. By minimizing the need for intermittent fluid changes and decreasing the number of chest drains needed, the initial preservation of a potential DCD could even be performed in a less specialized environment, allowing emergency departments in smaller hospitals to participate in DCD. In addition, this simplified technique allows the next-of-kin to spend more undisturbed time with the donor, as no interventions are needed for lung preservation purposes during the first hour after death.

Table 8. Lung function parameters during EVLP.

	Standard		Simplified		*p*-value
	Median	Range	Median	Range	
PaO_2					
FiO_2 - 0.5	31	24–36	29	16–34	0.75
FiO_2 – 1.0	72	68–81	67	48–74	0.75
FiO_2 – 0.21	14	12–17	13	12–14	0.63
$PaCO_2$					
FiO_2 – 1.0	4.7	4.0–5.4	4.5	4.2–4.9	0.25
ΔPaO_2					
FiO_2 – 0.21	-1.0	-4.2–1.3	-1.5	-2.3–0.9	0.69
PVR					
FiO_2 – 1.0	740	500–890	657	420–2700	0.75
Compliance					
FiO_2 – 1.0	39	26–87	37	21–59	0.63
Wet/Dry ratio					
	5.7	4.9–6.7	6.1	5.2–7.2	0.70

No significant differences were noted between the groups for: PaO_2 (kPa); $PaCO_2$ (kPa); PVR (dynes*sec/cm5); compliance (ml/cm H_2O); or wet/dry ratio. ΔPaO_2 indicates the difference in PaO2 between *in vivo* and *ex vivo* recordings in the specific animal.

Summary

Taken together with previous studies on the preservation of lung grafts inside the donor after uncontrolled DCD, Papers I and II provide support for a "hands-off period" of 1 hour after the declaration of death. In addition, Paper II supports the use of a simplified technique for *in situ* cooling of the lung graft. Combined with EVLP for the evaluation of DCD lung graft function, this is the foundation for the introduction of a clinical lung transplantation program with uncontrolled DCD lung donors.

EVLP in clinical lung transplantation

As outlined in the *Introduction*, a high percentage, in some regions up to 85%, of lungs from DBD are discarded due to known or presumed organ dysfunction (7), meanwhile a few regions having exceptionally high rates of up to 50% acceptance of donor lungs. (79). Distinguishing donor lungs that are stably dysfunctional from those that show reversible dysfunction is a frequently encountered dilemma for transplantation teams. Unwillingness to risk transplanting a recipient with a non-functioning lung will often lead the team to adopt a conservative strategy that involves rejection a marginal donor lung.

EVLP has the advantage of offering a functional test of the donor lung *ex vivo* under the control of the transplantation team. The EVLP method also has the potential to improve organ function through various treatments. Already before the use of EVLP in clinical transplantation, it was suggested that more than 40% of rejected donor lungs could be suitable for transplantation (80). Steen et al. (19) introduced EVLP as part of clinical lung transplantation by performing a quality test and transplanting a lung from a DCD (19). The EVLP protocol was later expanded in Lund to include DBD lungs that had been turned down for transplantation due to suspected impaired function (26). EVLP of suboptimal donor lungs was subsequently refined and implemented on a large scale by the Toronto transplantation group, as reported by Cypel et al. (81).

At SU, EVLP was introduced into clinical practice in 2011. The first transplantation of a lung after EVLP was performed in January 2011. The initial protocol used was a slight modification of the method proposed by the Lund group. While many investigators have performed clinical studies on EVLP of rejected door lungs, only a few have proceeded to transplant the evaluated lungs (Table 9).

Table 9. Published studies of clinical EVLP followed by transplantation into recipients.

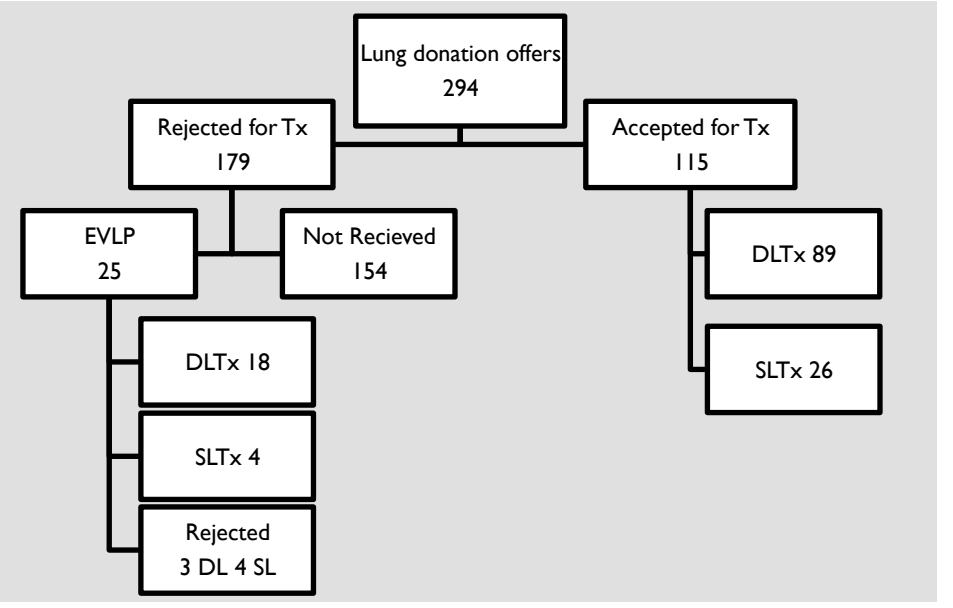
Reference	EVLP inclusion criteria	No. of EVLP	Donor P/F ratio	EVLP criteria for transplantation	EVLP P/F ratio	No. of transplanted EVLP lungs	Outcomes	Comment
Aigner C 2012 **(37)**	P/F ratio <40 kPa	13	28.8	P/F ratio PV-PA >47 kPa	62.1	9 (69%)	PGD >1 at 72 hours, 0; 30-day mortality, 0%	Trauma lungs did not improve with EVLP
Boffini M 2013 **(82)**	N/A	9	N/A	N/A	N/A	7 (78%)	N/A	Very limited data
Valenza F 2014 **(83)**	P/F ratio < 40 kPa or unclear function	8	35.2	N/A	69	7 (88%)	60-day survival, 100%. 30 day survival, 100%	Few EVLP data presented
Cypel M 2012 **(81)**	P/F ratio <40 kPa or x-ray edema or low lung compliance or DCD	58	44.5	N/A	68.4	50 (86%)	PGD >2 at 72 hours, 2%; 30-day mortality, 4%; 1-year survival, 86%	22 pairs of evaluated lungs from DCDs
Ingemansson R 2009 **(26)**	P/F ratio <40 kPa	9	22.8	P/F ratio >50 kPa	68.7	6 (67%)	1-year survival, 67%	EVLP with red blood cells
Zych B 2012 **(84)**	"Low" P/F ratio or abnormal x-ray	13	42	P/F ratio >50 kPa	67	6 (46%)	Mean ventilator time, 263 hours; 3-month survival, 100%	Two patients on ECMO after procedure
Wallinder A Paper V	P/F ratio <40 kPa / x-ray pathology / uncertain function	25	28.2	P/F ratio >40 kPa	63.7	22 (88%)	1-year survival, 89%	Paper IV

A total of 107 EVLP transplantations including previously unreported data from paper V. P/F ratio, PaO2/FiO2 Ratio; N/A, No available data; PGD, Primary graft dysfunction; ECMO, Extra corporeal membrane oxygenation.

Selection of lungs for EVLP

The selection criteria for donor lung eligibility for the EVLP procedure vary among clinics. The Lund group and Aigner and co-workers have reported using lungs for EVLP that have P/F ratios <40 kPa but that otherwise fulfill the normal ISHLT criteria (26, 37). Zych and co-workers did not define precisely their criteria for the P/F ratio other than describing it as "low" (84). Some groups consider an abnormal chest x-ray as an additional inclusion criterion. In the Toronto group, a P/F ratio <40 kPa is used as an inclusion criterion for DBD lungs. In addition, donor lungs with radiologic signs of pulmonary edema, poor lung deflation, transfusion of the donor with more than 10 units of blood, and lungs originating from DCD group III donors can be subjected to EVLP (38). In a presentation at the ISHLT meeting in Prague in 2012, Dr. Keshavjee from the Toronto transplantation group stated "any lung that you are not comfortable to use based on donor assessment alone is a candidate for EVLP in the Toronto transplantation program".

Figure 8. Flow diagram showing the fates of donor lungs offered to SU in the period 2011–2013.



At SU, the criteria that were initially used for EVLP of donor lungs were one or both of the following:

- P/F ratio <40 kPa
- x-ray findings consistent with pulmonary edema

The criteria were soon thereafter expanded to include:

- Donor lungs for which the level of function was impossible to evaluate, i.e., the donor on ECMO;
- Donor lungs with acceptable function but probability of organ dysfunction due to i.e., pulmonary embolism or severe trauma as causes of donor death.

Once the EVLP program was started at SU, it was soon discovered that the criteria used for the selection of donor lungs for EVLP provided a basis for discussion among colleagues rather than a strict set of guidelines for EVLP inclusion. In the same way as the ISHLT criteria provide a general recommendation for the use of lungs from multi-organ donors, the criteria for EVLP provide a basis for discussion. Statements issued by representatives of the Toronto group and the vague inclusion criteria used in other clinical EVLP programs indicate that a very rigid inclusion process is inefficient and does not serve the clinical needs in reality. Essential exclusion criteria, such as infection with HIV or hepatitis, are of course unaffected by EVLP, as is a donor history of heavy smoking or malignancies. The selection workflow used for donor lung management in the period 2011–2014 is described in Figure 8. The characteristics of donor lungs that were rejected for transplantation but selected for EVLP are listed in Table 10.

Table 10. Characteristics of donor lungs (n=25) rejected for transplantation but selected for EVLP at SU in the period 2011–2014

Parameter	Median	Range
Age (years)	52	15–68
Cause of death		
Subrachnoidal hemorrhage	10	
Traumatic brain injury	3	
Anoxic brain injury	11	
Pulmonary embolism	1	
PaO_2/FiO_2 ratio (kPa)*	28.2	8.0 – 57.0
Radiographic findings		
Edema	4	
Spread Infiltrates	7	
Localized infiltrates	9	
No pathology	3	
Missing	2	

* P/F ratio of donor lungs at the time of acceptance for EVLP. No reliable blood gas analysis results were available for the two donors on ECMO.

Arterial blood gases are often considered to represent the most important factor for selection of the lung donor. The P/F ratio is also stipulated as an inclusion criterion in all but one of the reports presented in table 9. However, the predictive value of the donor P/F ratio for graft survival in the recipient post-transplantation has been questioned (11). EVLP evaluation of lungs from controlled DCD is used at some, but far from all transplantation centers. As mentioned earlier in this thesis, DCD is not allowed in Sweden. In the experience of the Toronto group, lungs from controlled DCDs are included. These donors do not *per se* have inferior lung function. On the contrary, it has been proposed that DCD lungs may be a better substrate for transplantation, as the process of lung destruction that occurs in conjunction with a fatal brain injury are absent in the donor from DCD. The length of ICU stay may also be reduced, thereby reducing both the harmful effects of prolonged mechanical ventilation and the risk of bacterial infections. To date, no study has been published that provides evidence for improved recipient outcome if EVLP is used in controlled DCD, or for that matter in DBD.

The EVLP procedure

The EVLP strategy is described in Papers III–V and in the respective sections earlier in this thesis. In summary, the perfusion was performed with the Vivoline LS1 (Vivoline AB, Lund, Sweden). The perfusate consisted of Steen Solution (XVIVO AB) mixed with red blood cells to a hematocrit (hct) of 10%–15%. The lungs were warmed, and the flow rate was slowly increased to a maximum of 70 ml/min/kg donor body-weight. The pulmonary artery (PA) pressure limit was gradually increased to 20 mmHg. Mechanical, volume-controlled ventilation with a positive end-expiratory pressure (PEEP) level of 5 cm H_20 and a tidal volume of 6 ml/kg donor body-weight were applied. At 36°C, an incremental PEEP trial was performed to recruit atelectatic lobes/segments. The oxygenator was thereafter used to deliver CO_2 to the perfusate. Repeated blood samples for gas analysis was drawn from the left atrium and compared to simultaneous samples from the pulmonary artery blood. The deoxygenated blood in the pulmonary artery never exceeded a PO_2 value of 7 kPa. The delta values were not calculated. A collapse-test was performed at a FiO_2 value of 1.0 by disconnecting the tracheal tube at the end of inspiration. Recoil of the lungs was evaluated subjectively. The pulmonary mechanics, physiologic dead-space fraction (calculated as $PaCO_2$ – $EtCO_2$ / $PaCO_2$), static lung compliance, PVR were continuously monitored.

Acceptance of EVLP-evaluated lung(s) for transplantation was based on the following criteria: a) a lung oxygenation capacity with a P/F ratio >40 kPa during the evaluation phase; b) stable hemodynamic and respiratory variables (PVR, peak airway pressures, and lung compliance) during EVLP. Although no absolute cut-off levels were established, the measurements were compared to normal physiologic

ranges. A negative trend during EVLP, with the quality of the recordings deteriorating, was considered a contraindication for transplantation; c) the absence of macroscopic signs of pneumonic infiltrates or lung infarctions; and d) a normal collapse test. Accepted lungs were cooled in the EVLP system. The ventilator was disconnected at 32°C. The perfusate flow through the lungs was exchanged for surface cooling at 12°C. The EVLP perfusate was then flowing over the compress-embedded lungs and maintained at 8°C. In the case of DLTx, lungs were split and the second lung was kept cold by continuous surface cooling as the first lung was transplanted.

Table 11. EVLP data for lungs selected for transplantation

EVLP variables (n=22)	Median	Range
PaO_2 (kPa) at FiO_2 of 1.0	63.7	38.5–79.2
PAwP (mmHg) at FiO_2 of 1.0	13	10–20
Compliance at FiO_2 of 1.0	65	49–104
PaO_2 (P/F) improvement from donor to EVLP (kPa)*	29.7	0–50.7
PVR (dynes × s × cm^{-5}) at FiO_2 of 1.0	385	240 – 667

* One donor was on ECMO and was therefore excluded from the median value for this parameter. EVLP, *Ex vivo* lung perfusion; P/F ratio, PaO_2/FiO_2; PAwP, peak airway pressure; DSF, dead-space fraction; PVR, pulmonary vascular resistance.

The lungs from 25 donors were subjected to EVLP. Three double lungs and four single lungs were deemed unsuitable for transplantation due to impaired blood gas function or based on abnormal lung pathology, such as hematoma or consolidation. One recipient had end-stage pulmonary fibrosis and was critically ill, and since the thoracic cavity was very small, bilateral upper lobectomies were performed on the donor lungs during EVLP before transplantation. The EVLP data for lungs selected for transplantation are listed in Table 11.

In Paper V, 17 DLTx, 1 bi-lobar transplant, and 4 SLTx were performed after EVLP and compared with 89 DLTx and 26 SLTx in the conventional group (Table 10).

Minor modifications have been made during the course of the EVLP program. For the early reconditioning phase of the EVLP, we have over time adopted a more careful ventilation and perfusion strategy. Nevertheless, for the EVLP evaluation, we believe that the conditions accurately mimic the *in vivo* environment in which the lung must function after transplantation. An algorithm that includes cut-off values that could be used to select the optimal lungs for transplantation after EVLP is currently lacking. From our initial experiences with EVLP, we conclude that the

macroscopic appearance of the lung and the P/F ratio remain as the parameters that have the highest impact on lung function after transplantation. In borderline cases, such as the one described in the example below (text box), other data obtained during the EVLP, such as compliance and dead-space fraction, can provide supporting evidence for accepting or rejecting the donor lungs.

EVLP Case

One of the lungs in the EVLP group was donated by a previously healthy 19-year-old man who committed suicide by hanging. The lungs were assigned to EVLP as the chest x-ray and the clinical investigation showed suspected aspiration of gastric contents. During EVLP, the lungs were considered borderline for transplantation, with P/F ratios of 56 kPa for the left lung and 21 kPa for the right lung. During evaluation a PEEP of 7 cmH_2O was applied instead of 5 as stated in the protocol. We proposed that the left lung function would be sufficient and provide time for the right lung to heal. The static lung compliance was 52 ml/cm H_2O. Both lungs were transplaned and the recipient was extubated after 24 hours, but re-intubated after 5 days. Lung function steadily worsened and the patient needed veno-venous ECMO at postoperative Day 14 and was re-transplanted at day 22. Computed tomography scans revealed widespread and severe bronchiectasis. Cultures from the donor lungs grew drug-resistant fungi. The cause of the failing lung function could have been related to the donor, the EVLP, or the postoperative period, although it was most likely due to airway trauma in the donor in conjunction with aspiration of gastric contents.

In a retrospective analysis of the described case, rejection of the lungs for transplantation based on the EVLP data provided, appears to have been been the correct decision, and highlights the importance of taking all EVLP parameters in consideration before one proceeds to transplant the lungs.

A P/F ratio >40 kPa is most likely sufficient for adequate lung function after transplantation if the complementing EVLP data (PVR, compliance, macroscopic appearance) are within the expected limits. As experience with EVLP increases, our ability to select or reject the most suitable lungs for transplantation will undoubtedly improve. In this respect, the outcomes for the recipients of EVLP-treated lungs should also improve. The optimal yield of transplantable lungs after EVLP depends on the inclusion criteria for EVLP and the experience of the transplantation team. Rates of 46%–100% have previously been reported (26, 37, 81). In our series, the turnover rate after EVLP was 88%, a percentage that was boosted by the use of single lungs in four cases (Figure 8)

Short-term results after transplantation of lungs following EVLP

Both the short-term and long-term outcomes are dependent upon which EVLP lungs that are selected for transplantation and also upon the status of the lung recipients. For example, in Paper V, a large proportion of the EVLP group had the preoperative diagnosis of pulmonary fibrosis, whereas almost 10% of the recipients in the conventional group were on ECMO at the time of transplantation. Lung function in the recipient is usually evaluated based on the length of time on the ventilator, early graft function, and the length of stay in the ICU.

Time to extubation

The lung recipient is transported to the ICU while intubated with ventilator support. When respiratory function is deemed adequate, with respect to oxygenation capacity and the need for positive end-expiratory pressure, the recipient is a candidate for extubation. Inferior graft function will prolong the time on ventilator support. Of course, other factors also affect the decision to extubate. In the largest published study from the Toronto group (n=50), the median time to extubation was 2 days (range, 1–101 days) (81). In corresponding data from Aigner et al. (37) and Zych and al. (84), the times to extubation were 48 hours and 214 hours, respectively. In the latter publication, 2/6 recipients required ECMO postoperatively. In Paper IV, the 11 recipients of EVLP-treated lungs had a median time on ventilator of 12 hours, which is similar to times given in the other EVLP studies but significantly longer than the 6 hours observed for our own control group (p=0.05). In Paper V, the EVLP group grew to include 22 transplanted patients. Thus, the median time to extubation decreased to 7 hours, as compared with 6 hours in the control group (p=0.26).

Duration of stay in the ICU

The length of time that lung recipients need to stay in the ICU is also dependent upon multiple factors. The preoperative state of the recipient is a significant contributor. A patient who is treated with ECMO before transplantation is often in a bad clinical state and will likely spend longer time in the ICU postoperatively than a patient who is awaiting an LTx at home. The Toronto group reported (81) a median ICU stay of 4 days with a range of 1–100 days, the latter reflecting the heterogeneity of this patient group. The corresponding median ICU stay data from Aigner et al. (37) and Zych et al. (84) were 5.5 days and 20 days respectively. The longer ICU stay in the report from Zych et al. indicates a high complication rate. In Paper IV, the recipients of rejected donor lungs transplanted after EVLP had a median postoperative ICU stay of 152 hours (range, 40–625 hours) versus 48 hours (range, 22–1632 hours) in the control group (p=0.01). In the larger cohort in Paper V, the median ICU stays were 3 days (range, 1–39 days) in the EVLP group and 2

days (range, 1–60 days) in the control group (p=0.06). The lung injury inflicted in the donor, often accompanied by pulmonary edema, is in our experience not completely resolved during EVLP, but is in the recipient. Therefore, it is reasonable to expect somewhat longer ventilation times and ICU stays in the EVLP group. We argue that EVLP provides a tool for selecting potentially good lungs amongst the initially rejected lungs, although it does not *per se* reverse donor lung pathology, other than reducing atelectasis and secretions that obstruct the bronchi.

Primary graft dysfunction

Primary graft dysfunction (PGD), which is a type of acute lung injury that is similar to acute respiratory distress syndrome (ARDS), occurs within 72 hours of the transplantation (85). Inflammation leads to increased permeability of the lung capillaries and results in alveolar edema. In DBD lung transplantation, PGD affects 11%–25% of recipients and is the leading cause of early mortality (86, 87) The PGD scoring system is analogous to that used for ARDS and consists of a composite of the P/F ratio and x-ray findings of edema (Table 12). While PGD is primarily a measure of early graft failure, it also predicts later occurrence of rejection (88). The incidence of primary graft dysfunction higher than grade 1 at 72 hours was similar in the EVLP group (9%) and the control group (8%) in Paper IV, and also in the larger cohort described in Paper V (13.6% in the EVLP group versus 10.7% in the control group). When our population is compared to the data from Cypel et al. (81), the incidence of severe PGD seems higher in our cohort. However, the donors in our study were older, which is a risk factor for PGD, and the donor P/F ratios were also worse.

Table 12. Classification of primary graft dysfunction.

Grade	P/F ratio	Radiographic infiltrates/ Pulmonary edema
0	>40	Absent
1	>40	Present
2	27–40	Present
3	<27	Present

Early morbidity and mortality

One patient in the EVLP group died in the operation room during the lung transplantation procedure due to bleeding complications not related to the lung graft. Another patient in the EVLP group was re-transplanted early due to graft failure (case presented in text box p.45). This patient and the other 20 patients in the EVLP group were discharged alive from hospital giving a 30-day survival of 96% in Paper V. Cypel et al. (81) have reported a 30-day survival rate of 96% after EVLP transplantation.

In summary, early results indicate that the length of ICU stay seems longer for recipients of EVLP-treated lungs. The time on ventilator support and the incidence of PGD are however similar for EVLP lung transplantation and conventional lung transplantation. Overall, we believe that these results encourage rather than limit the use of EVLP-evaluated donor lungs.

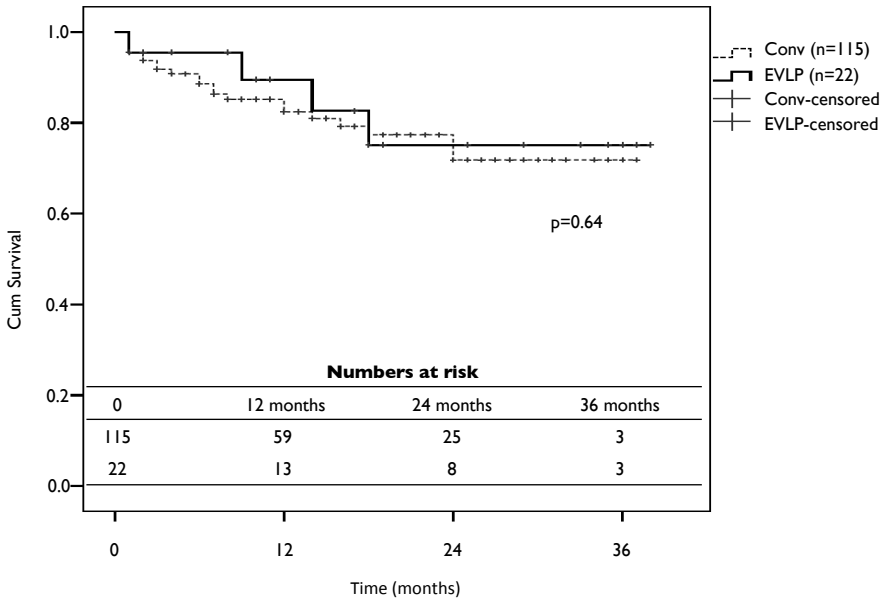
Long-term results after transplantation of lungs following EVLP

Long-term follow-up data after EVLP transplantation are sparse. Cypel et al. (81) have reported a 1-year survival rate of 87% after EVLP. The 3-year survival rate reported by the same group is 70%, although the number of patients who survived this long is not outlined, and they were probably few. In the report of Aigner et al. (37), the 1-year survival rate is 78% (7/9 patients). No chronic rejection was diagnosed in the remaining patients. Zych et al. (84) reported a 3-month survival rate of 100% (6/6 patients); one patient died after 7 months and the 1-year survival data are missing. Ingemansson et al. (26) showed 1-year and 2-year survival rates of both 67% (4/6 patients)

In Paper IV, which includes the first 11 recipients of EVLP lungs at our center, all 11 patients were discharged alive from hospital. At 3 months of follow-up, the FEV 1.0 was similar for recipients of EVLP lungs and conventional lungs. In Paper V, the initial 22 patients are reviewed. The cumulative 1-year survival rate was 89% in the EVLP cohort and 82% in the control group. The cumulative 2-year survival rates were 75% and 72%, respectively (Figure 8). It is notable that only 8 patients in the EVLP cohort yet have reached 24 months, and that the data must therefore be interpreted with great caution.

The survival of the relatively small EVLP group seems non-inferior to that of the conventional group. When compared to the ISHLT data (5), the survival rates of both the EVLP and the control group are well in line with what would be expected.

Figure 9

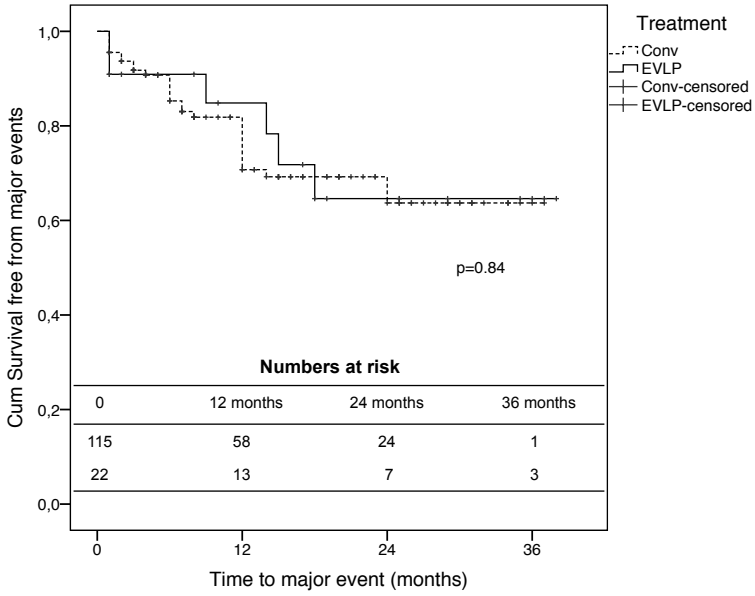


Comparisons of the cumulative survival rates of recipients of lungs with prior EVLP evaluation (EVLP) and consecutive recipients of standard donor lungs (Conv) during the same time period (p=0.64). Source: Paper V.

Chronic lung allograft dysfunction, defined as bronchiolitis obliterans syndrome (BOS) (i.e., a decrease in FEV 1.0 to less than 80% from the best post transplant recordings) (89) or as restrictive allograft syndrome (RAS) (i.e., TLC <90% of baseline and radiologic signs of progressive fibrosis) (90), occurs frequently in the lung-transplanted population. In Paper V, the comparable event-free survival, defined as survival without re-transplantation, RAS or BOS is demonstrated between the groups. BOS or RAS was diagnosed in 2 patients (9%) in the EVLP group and 17 patients (15%) in the control group during the study period (Figure 9).

In conclusion, data from the follow-up of recipients of EVLP-treated lungs at both our own center and other centers show similar survival and complication rates for recipients transplanted with conventional or EVLP-treated lungs. During the study period, the acceptance rate at our center was 39% for lungs without prior EVLP, which is a high percentage compared to what other institutions have reported. Moreover, of the rejected 145 donor lungs 25 pairs were subjected to EVLP and 22 patients subsequently transplanted with EVLP-evaluated lungs. This implies that with an EVLP program, up to 50% of lungs from organ donors can be utilized.

Figure 10



Comparisons of the cumulative major adverse event-free survival rates for recipients of lungs with prior EVLP versus consecutive recipients of standard donor lungs during the same time period (p=0.84). Major adverse events are defined as death, re-transplantation, RAS or BOS. Source: Paper V.

This finding would have an even greater impact in centers that have a higher rate of declined lungs. The results from these few non-randomized studies cannot be compared to the results of a randomized trial and should there for be assessed cautiously, since they likely are insufficiently powered to allow a true comparison of the groups.

Future and on-going trials

Several institutions have on-going clinical trials of EVLP. The INSPIRE trial (clinicaltrials.gov NCT 01630434) is an on-going, randomized study comparing cold static preservation of donor lungs to lung perfusion with a portable EVLP unit. This method allows for EVLP preservation from the donor hospital up until transplantation. The INSPIRE study does not focus on donor lungs with suboptimal function, but instead focuses on organ preservation, and therefore is of less interest in this context. The first 12 transplantations in this trial were reported in 2012 (24). The NOVEL trial (clinicaltrials.gov NCT 01365429) is an on-going, multicenter US study. Standard donor lungs (n=42) are compared to extended-

criteria donor lungs (n=42) transplanted after EVLP according to the Toronto protocol. Recruitment for this study is estimated to close in May 2014. The DEVELOP-UK trial (controlled-trials.com ISRCTN 44922411) is another on-going trial comparing standard donor lungs with extended-criteria donor lungs (ratio of 3:1). The investigators in this study apply an EVLP protocol similar to that used in Papers III–V, and they have wisely chosen 1-year survival as the primary end-point. Finally, the EXPAND trial (clinicaltrials.gov NCT 01963780) is a planned study to investigate and promote a specific lung perfusion device. Extended criteria donor lungs are proposed to be included, although no control group of standard donor lungs is included.

As stated previously, data on long-term follow-up after EVLP transplantation are sparse. However, it has been proposed that EVLP could decrease the incidences of both PGD and BOS/RAS. Apart from the obvious benefit of excluding lungs with inferior function detected during EVLP evaluation, it has been suggested that passenger leukocytes, which could contribute to allo-reactions, would be removed during EVLP, thereby reducing the risk of rejection. An "all-comer" study of donor lungs, both standard and marginal ones, with PGD as the primary end-point and BOS/RAS as the secondary end-point, could potentially demonstrate a reduced severity of immune reactions to the graft.

Key results and final comments

Papers I and II

Lung donation after uncontrolled DCD has a tremendous potential for future transplantation activities. Optimal preservation of the lungs *in situ* remains to be elucidated. In Paper I, we show that Heparin administered post-mortem and followed by chest compressions is of no benefit when donor lung function is evaluated during EVLP. In Paper II, cooling of the donor lungs in the donor is investigated. We show that a simplified, less-invasive preservation technique is sufficient for adequate donor lung preservation. The results from Papers I and II indicate that all invasive procedures can be avoided in the potential DCD donor during the first hour after death, and that preservation during the first 3 hours after death can be limited to just a bilateral chest drain and one cold intrapleural flush. These findings mean that the donation process is greatly simplified. The clinical implication is that a less-invasive management of the uncontrolled donor will provide more undisturbed time for the next-of-kin to spend with their deceased relative. It may also result in the acceptance of DCD among hospital personnel working in the Emergency departments.

Papers III–V

Transplantation teams discard up to 85% of all the lungs from organ donors on the assumption of organ dysfunction. EVLP provides a tool for identifying and reconditioning of suboptimal donor lungs. During controlled perfusion and ventilation, members of the transplantation team can perform an assessment of the lung function. In Paper III, we describe the EVLP strategy introduced at our center. We focus on the technical aspects, as well as the EVLP results from our early experience. In Papers IV and V, we review our EVLP experiences with the first 22 recipients of EVLP lungs and compare them to 122 consecutive recipients of non-EVLP lungs. In the first analysis (Paper IV), patients who were transplanted with EVLP lungs had longer ventilator and ICU times. This difference was not replicated as our experience of EVLP increased (Paper V). Hospital stay, long-term survival, and occurrence of chronic rejection did not differ between the groups.

Final comments

The number of available donor lungs does not meet the demand for such organs. Consequently, many patients with end-stage lung disease die prematurely. Donation after circulatory death (DCD) could dramatically increase the number of available donor lungs. The donation process must be simplified to create acceptance among the public, the next-of-kin, and hospital personnel for this form of organ donation. The DCD concept investigated in this thesis, using a less-invasive preservation regime followed by EVLP evaluation, provides a solid foundation for the introduction of uncontrolled DCD in the clinical setting. The time is ripe for the introduction of DCD lung transplantation in Sweden.

We have demonstrated that EVLP evaluation of initially rejected donor lungs can be performed safely and make a significant contribution to a lung transplantation program without compromising the outcomes for recipients. With the implementation of a well-functioning EVLP regime, up to 50% of the lungs obtained from multi-organ donors could be used safely. The introduction of an EVLP program could have a massive impact if applied in centers with low percentages of donor lung acceptance. Future studies should focus on improved methods for the reconditioning and treatment of donated lungs using EVLP. EVLP can also serve as a platform for advanced repair of the injured donor lung.

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Populärvetenskaplig sammanfattning

Lungtransplantation är en livsförlängande ingrepp för personer med mycket svår lungsjukdom. I Sverige, liksom i resterande delar av världen, begränsas dock antalet utförda lungtransplantationer av bristen på donerade organ. Förutom opinionsbildning och information, syftande till att öka antalet individer positivt inställda till att donera, finns det andra möjligheter att öka antalet transplanterbara lungor. Om dessa möjligheter handlar denna avhandling.

Historiskt genomfördes de första lungtransplantationerna med organ donerade från patienter avlidna till följd av hjärtstillestånd. Tillkomsten av ”hjärndödsbegreppet” i Sverige 1987 ändrade detta paradigm. En hjärndöd patient har en omfattande och oåterkallelig hjärnskada som inte är förenlig med liv. Hjärtat slår dock och uppehåller cirkulationen medan en respirator sörjer för andningen. Hjärndödsbegreppet ökade tillgången på donatorer och möjliggjorde att lungfunktionen kunde utvärderas i donatorn. Sedan slutet av 1980-talet transplanteras i Sverige uteslutande lungor donerade från hjärndöda men med pågående cirkulation. I syfte att utöka antalet tillgängliga organ för transplantation har de senaste 10 åren organdonation från människor avlidna till följd av hjärtstillestånd åter aktualiserats. Begreppet hjärtdöd är inte unisont definierat men innebär i praktiken ett oåterkalleligt hjärtstillestånd som i sin tur medfört hjärndöd.

I samband med ett hjärtstillestånd drabbas kroppens alla organ av snabbt inträdande syrebrist (ischemi). Upprepade studier har dock påvisat lungans unika förmåga att motstå ischemiska skada. Ett flertal metoder har förespråkats för att optimalt bevara lungornas funktion i en hjärtdöd organdonator under ischemi. För att förhindra blodproppar har man gett en injektion med det blodförtunnande medlet Heparin till den hjärtdöda donatorn. Då läkemedelet administreras efter det att cirkulationen upphört utförs också ytterligare hjärtkompressioner på den avlidna patienten för att distribuera läkemedlet till lungorna. När patientens eller anhörigas positiva inställning till donation är känd kyls lungorna i donatorns kropp med kall lösning via dränage in i brösthålan. Tiden fram till att kylningen startas är kritisk då de varma lungorna oundvikligen drabbas av cellskador. När organen väl är kylda bromsas cellernas metabolism och därmed cellsönderfallet. Innan transplantation av

lungor från en hjärtdöd donator sker, måste det säkerställas att organens funktion är tillräcklig för att fungera i recipienten.

Alla ingrepp som måste utföras på en hjärtdöd, potentiell donator medför ett etiskt och logistiskt dilemma. Sjukvårdpersonal förväntas övergå från att försöka rädda ett liv till att vidmakthålla funktionen (preservera) i ett organ. Åtgärder såsom att återuppta hjärtmassage på en person, som nyligen dödförklarats, medför att diskussioner kring etik och behandlingsprinciper uppstår. Man kan befara att benägenheten hos sjukvårdspersonalen att initiera processen kring organdonation kan minska i relation till hur många och hur komplicerade åtgärder som behöver utföras på den avlidne.

I delstudierna I och II undersöktes i en stordjursmodell preservation av lungor hos hjärtdöda, potentiella donatorer. Ambitionen var att experimentellt visa på moment som kan uteslutas eller förenklas i hanteringen av hjärtdöda donatorer och donerade lungor utan att detta påverkar organens funktion negativt. Efter att lungorna explanterats från donatorn utvärderades deras funktion i en för ändamålet, specialbyggd hjärt-lungmaskin. Detta benämns ex-vivo lungperfusion (EVLP). Lungorna försörjs med cirkulation från maskinen och ventilation via en respirator. Det blir på detta sätt möjligt att styra tryck och flöden i lungcirkulationen samt ventilation och syrgastillförsel via respiratorn. Lungornas cirkulation och gasutbytesförmåga kan utvärderas genom att observera flöden, tryck och genom att mäta nivåer av syre i den vätska som cirkulerar lungorna.

Resultaten från delstudie I och II gav tillsammans stöd för att avstå från alla ingrepp i organbevarande syfte i donatorn under den första timmen efter att döden inträffat. Delstudie I gav stöd för att utesluta administration av det blodförtunnade Heparinet och delstudie II visade att en förenklad metod för kylning av lungorna i donatorn kunde tillämpas. I klinisk praxis skulle detta kunna underlätta användandet av lungor donerade från hjärtdöda och medföra en förenklad donationsprocess samt i förlängningen en ökad tillgång på donerade lungor.

Internationellt tas idag färre än en tredjedel av hjärndöda organdonatorers lungor till vara för transplantation. Ofta är detta en följd av att donatorn anses för gammal för att man skall kunna använda dennes/as lungor, och eller att många års rökning har föregått dödsfallet eller att det finns annan misstanke om lungsjukdom. I många fall anses en nedsättning i donatorns lungfunktion dock enbart vara en följd av det som orsakat dödsfallet, ofta en omfattande hjärnskada. En sådan, hastigt påkommen, funktionsnedsättning i en tidigare frisk lunga skulle potentiellt gå att återställa. För att utvärdera och förbättra funktionen hos donerade lungor explanteras dessa från donatorn och kopplas till EVLP.

I delstudierna III-V beskrivs införandet av denna metod i vårt kliniska transplantationsprogram. Efter utförda pilotförsök har lungor som normaliserat sin

funktion under EVLP, transplanterats till väntande, svårt lungsjuka patienter. Denna transplanterade grupp har sedan följts och jämförts med recipienter av ordinära donatorslungor. Data från sammanlagt 22 patienter, transplanerade med EVLP-evaluerade lungor, har jämförts med resultat från 115 samtidiga, kontrollpatienter. Resultaten från dessa båda grupper skiljer sig inte åt avseende organfunktion, förekomst av komplikationer eller överlevnad och vi kan dra slutsatsen att antalet använda lungor från donatorer kan utökas med hjälp av EVLP utan att äventyra medicinska resultat. Antalet lungtransplanterade patienter har således kunnat ökas till följd av att EVLP introducerats i klinisk praxis.

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