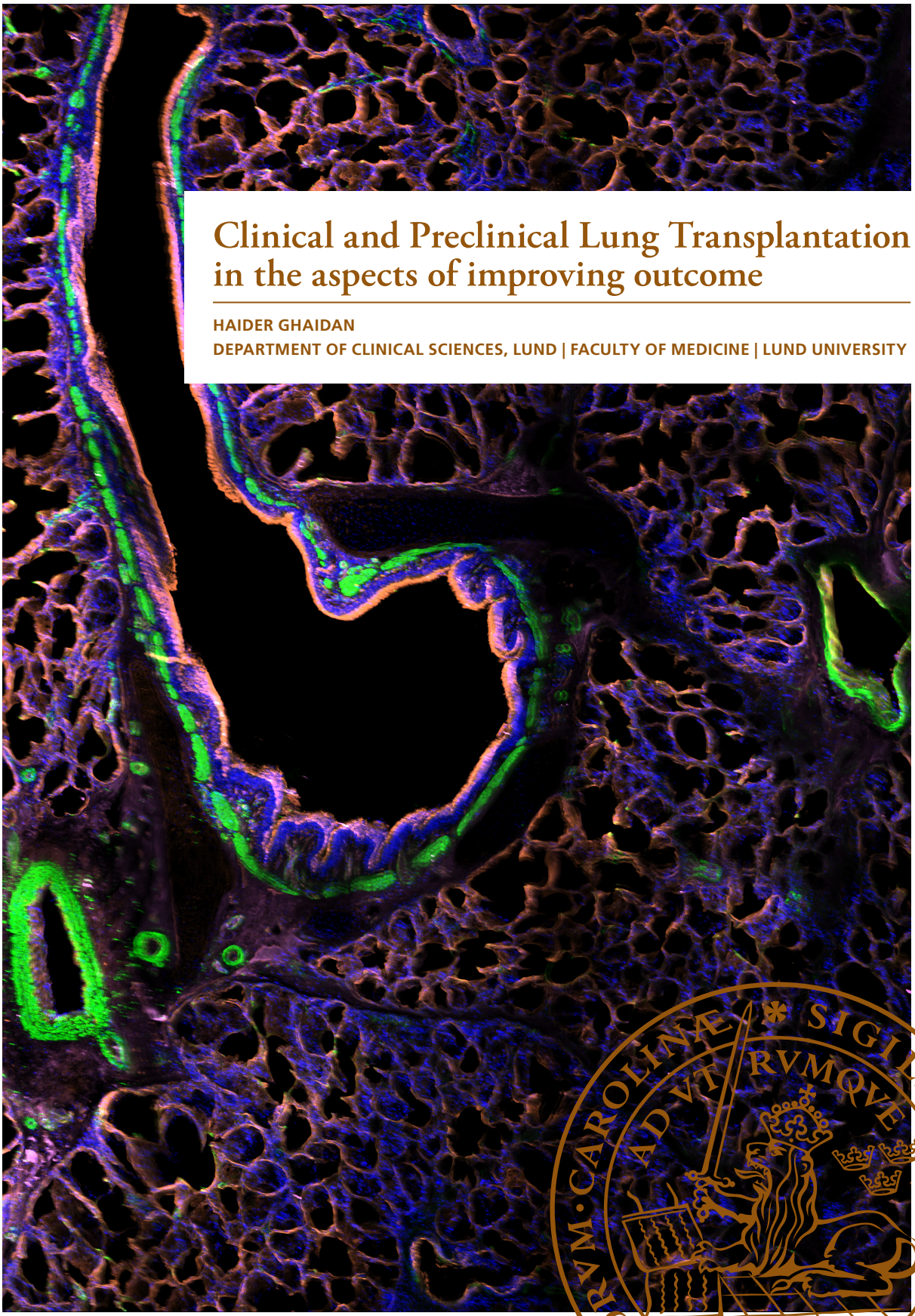
Clinical and Preclinical Lung Transplantation in the aspects of improving outcome

Ghaidan, Haider

2023

Document Version:
Publisher's PDF, also known as Version of record



Clinical and Preclinical Lung Transplantation in the aspects of improving outcome

HAIDER GHAIDAN
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

Clinical and Preclinical Lung Transplantation in the aspects of improving outcome

Haider Ghaidan MD

LUND UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.

To be defended at Föreläsningssal 4, Klinikgatan 21, Lund. Friday 10 March 2023 at 09:00.

Faculty opponent

Laila Johansson Hellgren

Associate Professor, Senior Consultant in Thoracic Surgery

Akademiska Hospital, Uppsala University, Sweden

Examining committee

Ellen Ostenfeld MD, PhD, Associate Professor, Skåne

University Hospital, Lund IKVL, Lund University

Peter Hasse Møller-Sørensen MD, PhD, Associate Professor

Rikshospitalet Copenhagen, University of Copenhagen, Denmark

Carl Jorns MD, PhD, Associate Professor

Department of Transplantation Surgery,
Karolinska Institute, Sweden

Organisation LUND UNIVERSITY Faculty of Medicine	**Document name** Lund University, Faculty of Medicine Doctoral Dissertation Series 2023:30
Department of Clinical Sciences, Lund Cardiothoracic Surgery	**Date of issue** **2023-03-10**
Author: Haider Ghaidan MD	Sponsoring organisation

Title and subtitle Clinical and Preclinical Lung Transplantation in the aspects of improving outcome

Abstract

Background

Lung transplantation (LTx) is an established therapeutic option for end-stage pulmonary disease. However, it remains restricted by donor lung scarcity. Donor's lungs are rejected frequently due to severe lung damage caused by aspiration or neurogenic pulmonary oedema that can all lead to acute lung injury (ALI), and more severe acute respiratory distress syndrome (ARDS). Lung transplant patients face poor survival rates in comparison with other solid organ transplantations. This is primarily due to a high incidence of postoperative complications, such as primary graft dysfunction (PGD) and chronic lung allograft dysfunction (CLAD), especially bronchiolitis obliterans syndrome (BOS).

The aim of this thesis was to expand the availability of a donor's lungs for transplantation. We sought to increase the chances of a lifesaving opportunity for recipients who may otherwise have remained on the transplant waiting list for years. We did this preclinically by utilising a variety of techniques to regain lung function in discarded lungs, thus increasing the donor pool. We investigated the role of cytokine adsorption during *ex vivo* lung perfusion (EVLP), and extracorporeal haemofiltration post-transplant as a means of treating and restoring the ARDS-damaged lungs and reducing the incidence of PGD post-transplantation. The lungs were evaluated regarding the development of primary graft dysfunction (PGD) in which cytokines seem to be an essential target given the outcome of significantly less PGD in the group receiving cytokine adsorption.

We suggest this treatment method will increase the availability of the donor's lungs and increase the tolerability of the donor's lungs in the recipient. The results of this study formed the basis for our idea to investigate the effect of mesenchymal stromal cell (MSC) therapy to restore gastric content aspirations damaged lungs and reduce the incidence of PGD at 72 hours' post-transplantation.

Furthermore, we explored pulmonary function, survival, and the incidence of CLAD between patients receiving marginal lungs after *ex vivo* lung perfusion (EVLP) reconditioning and patients receiving clinically standard lungs (conventional lungs) at our centre. These patients were followed for over 10 years. We did not find any difference in pulmonary function, survival, or incidence of CLAD, indicating that EVLP is safe to use and does not increase mortality.

We also explored the impact of allograft ischaemic time (IT) in lung transplantation survival rate which showed superior outcomes for IT between 120 and 240 minutes. Every 2-hour increase in IT was equivalent to an increased mortality of up to 24% within 5 years. This indicates that IT has a key role in improving LTx outcomes.

We explored the role of plasma biomarkers in the largest subgroup of CLAD, patients with BOS. Plasma from lung-transplanted patients with different BOS grades was analysed for protein biomarkers using Olink proteomics. A selective number of biomarkers were then validated using an enzyme-linked immunosorbent assay (ELISA) at baseline and after 1 year. Corticotropin-releasing hormone (CRH) levels were found to be related to different stages of BOS which identified CRH as a potential marker in a novel diagnostic tool to detect BOS.

In conclusion, using EVLP is a safe effective platform for cytokine adsorption therapy and MSC therapy which can restore pulmonary function in damaged donor lungs, thus increasing the donor pool. CRH is a novel potential biomarker in the progression of post-transplantation BOS grades.

Key words Acute lung injury. Primary graft dysfunction. Allograft ischaemic time. Bronchiolitis obliterans syndrome

Classification system and/or index terms (if any)

Supplementary bibliographical information	**Language** English
ISSN and key title 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2023:30	ISBN 978-91-8021-369-1

Recipient's notes	**Number of pages** 166	Price
	Security classification	

Signature  Date 2023-02-01

Clinical and Preclinical Lung Transplantation in the aspects of improving outcome

Haider Ghaidan MD

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Department of Clinical Sciences, Lund

Cardiothoracic Department

Supervisor: Professor Sandra Lindstedt, MD, PhD

Co-supervisor: Leif Pierre, PhD

Co-supervisor: Snejana Hyllén, MD, PhD

Cover photo: Lung parenchyma with fluorescence, taken by

Nicholas Bechet PhD (*Advanced Light Imaging Specialist*)

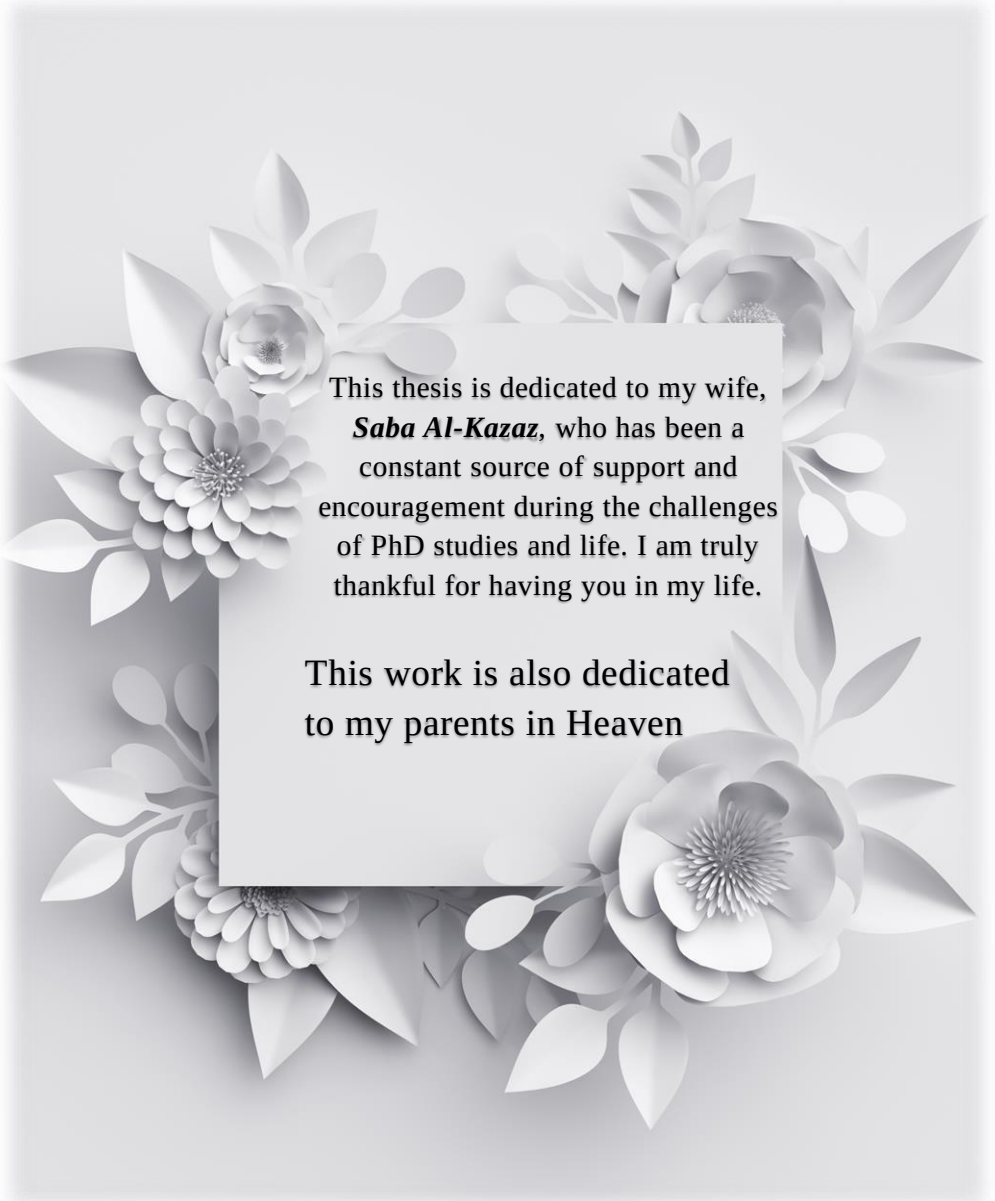
Lund University
Faculty of Medicine
Department of Cardiothoracic Surgery, Skåne University Hospital, Lund

ISSN 1652-8220
ISBN 978-91-8021-369-1

Printed in Sweden by Media-Tryck, Lund University
Lund 2023

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN



This thesis is dedicated to my wife, ***Saba Al-Kazaz***, who has been a constant source of support and encouragement during the challenges of PhD studies and life. I am truly thankful for having you in my life.

This work is also dedicated to my parents in Heaven

Table of Contents

1 List of publications

Paper I

Ghaidan H, Stenlo M, Niroomand A, Mittendorfer M, Hirdman G, Gvazava N, Edstrom D, Silva I, Broberg E, Hallgren O, Olm F, Wagner D, Pierre L, Hyllen S, Lindstedt S. Reduction of primary graft dysfunction using cytokine adsorption during organ preservation and after lung transplantation.

*Nature Communication*s. 2022; Jul 26;13(1):4173.

Paper II

Ghaidan H, Fakhro M, Andreasson J, Pierre L, Ingemansson R, Lindstedt S. Ten-year follow-up of lung transplantations using initially rejected donor lungs after reconditioning using *ex vivo* lung perfusion.

J Cardiothorac Surg. 2019;14(1):125.

Paper III

Ghaidan H, Fakhro M, Lindstedt S. Impact of allograft ischemic time on long-term survival in lung transplantation: a Swedish monocentric study.

Scand Cardiovasc J. 2020;54(5):322-9.

Paper IV

Niroomand A, Ghaidan H, Hallgren O, Hansson L, Larsson H, Wagner D, Mackova M, Halloran K, Hyllén S, Lindstedt S. Corticotropin releasing hormone as an identifier of bronchiolitis obliterans syndrome.

Sci Rep. 2022; May 19;12(1):8413.

Paper V

Ghaidan H, Olm F, Edstrom D, Mittendorf M, Niroomand A, Stenlo M, Hirdman G, Broberg E, Scheding S, Hyllen S, Pierre L, Lindstedt S. Live cell treatment in aspiration-induced donor lung injury in a transplantation model.

Manuscript. 2022.

2 Populärvetenskaplig sammanfattning (Summary in Swedish)

Lungtransplantation (LTx) är idag en väl etablerad medicinsk åtgärd för patienter med allvarliga lungsjukdomar som inte har något annat val vad gäller andra behandlingsalternativ. Ca 70 000 lungtransplantationer hos vuxna har rapporterats till internationella register fram till 2018 (ISHLT 2019). Antalet transplantationer årligen har ökat kontinuerligt sedan 90-talet och numera görs runt 4500 lungtransplantationer per år i världen. Trots ett ökat antal lungtransplantationer gör organbrist att behovet överstiger tillgången till transplantation.

Urvalet av patienter som lämpar sig för lungtransplantation är patienter som ska ha en kort förväntad överlevnad utan transplantation, men vara tillräckligt friska i övrigt för att ha en god förväntad överlevnad efter transplantationen. Dessa patienter ska uppfylla speciella kriterier innan de sätts upp på väntelistan, kriterierna finns beskrivna i internationella riktlinjer från International Society for Heart and Lung Transplantation från 2014. Ett av huvudkriterierna är att patienten har en förväntad livslängd på mindre än två år om man inte genomför en transplantation.

De vanligaste diagnoserna vid lungtransplantation är KOL/emfysem på rökbasis eller till följd av alfa-1- antitrypsinbrist, lungfibros, cystisk fibros eller pulmonell hypertension.

När man utför en lungtransplantation kan man antingen ersätta båda lungorna på en gång, så kallad dubbel lungtransplantation (DLTx), eller, som i några fall, endast ersätta en lunga, så kallad enkel eller singel lungtransplantation (SLTx). I de fall då patienten har uttalad hjärtsjukdom som kräver hjärttransplantation transplanteras både hjärta och lungor på samma gång, detta kallas hjärt- och lungtransplantation (HLTx).

En stor utmaning inom transplantationsmedicin är bristen på donerade organ. Behovet av donerade lungor är större än tillgängligheten, vilket medför att patienter tyvärr avlider medan de står på väntelistan för lungtransplantation.

Utöver bristen på donerade lungor kan dessutom endast 30–40 % av de potentiella donerade lungorna till slut användas för transplantation. Detta jämfört med att exempelvis cirka 80 % av donerade njurar används för njurtransplantation. Anledningen till att det blir en sådan låg andel lungor som accepteras för transplantation är tyvärr en följd av att den största delen av donerade lungor inte klarar av de kriterier som krävs för en transplantation.

År 2006 transplanterades för första gången i värden sex patienter med dubbel lungtransplantation med lungor som initialt var avvisade för transplantation på grund av att lungorna hade en låg syresättningsförmåga. Dessa lungor togs tillvara och behandlades utanför kroppen genom att använda ex vivo (utanför kroppen) lungperfusion (EVLP) som är en teknik för att utvärdera och behandla initialt avvisade donerade lungor, i förhoppningen att lungorna efter detta kan accepteras för transplantation. Ex vivo lungperfusion är alltså en maskin som stödjer lungorna utanför kroppen, även kallat rekonditionering. Denna maskin gör så att donerade lungor hålls ventilerade och cirkulerade med blod för att optimera förhållanden, samtidigt som lungorna behålls sterila. Maskinen bibehåller även lungans optimala luftfuktighet och temperatur under behandlingen. Tekniken är utvecklad av Professor Stig Steen och hans forskningsteam här i Lund.

I studie nr. 2 presenteras en 10-årsuppföljning av dessa ovan nämnda patienter som lungtransplanterades år 2006. Denna studie jämför resultaten mellan EVLP-behandlade lungor och standardlungor och visade inte någon större skillnad vad gäller den långsiktiga överlevnaden och lungfunktionen. Tekniken med EVLP används idag på många lungtransplantationskliniker över hela världen och anses vara överlägsen vad gäller utvärdering och rekonditionering av donerade lungor som initialt blivit avvisade för transplantation på grund av akuta lungskador så som acute respiratory distress syndrome (ARDS).

I studie nr. 1 presenterade vi en metod för behandling av lungskador orsakats av giftiga ämnen i blodbana. Genom att låta blodet i EVLP-kretsen cirkulera genom ett filter som adsorberar så kallade cytokiner kan man reducera graden av inflammation i den donerade lungan. Cytokinadsorbtion återställer lungfunktionen vilket i sin tur leder till att man kan acceptera fler lungor för transplantation och på samma gång reducera frekvensen av akut avstötning som fortfarande är den ledande orsaken till för tidig död och som bidrar till kronisk rejektion. Resultatet av denna studie låg till grund för vår idé till den studie nr. 5, vilken också är en djurmodell med lungskada orsakats den här gången av inandning av maginnehåll. Inandning av maginnehåll är

tyvärr vanligt i situationer som till exempel hjärtstillestånd och kan göra att lungorna på den avlidne inte går att donera för transplantation där vi behandlade lungorna med stamceller under EVLP samt efter transplantationen. Vår modell är den enda i världen som utför hemodynamiska mätningar under tre dagar efter lungtransplantation på grisar. Tack vare resultatet i dessa prekliniska studier har vi erhållit etiskt godkännande för en klinisk studie som vi tror kommer att vara av stor betydelse för patienter framöver.

Det största problemet med perioden som följer efter en lungtransplantation är risken för utveckling av kronisk rejektion. Detta är också den faktor som påverkar patienternas långsiktiga överlevnad mest. Kronisk rejektion har tidigare definierats som bronchiolitis obliterans syndrome (BOS) men definieras idag som chronic lung allograft dysfunction (CLAD) som har två underkategorier, en obstruktiv form (BOS) och en restriktiv form (restrictive allograft syndrome, RAS). CLAD är ovanligt under det första året efter lungtransplantation, men studier visar nu att mellan 45 - 75 % av alla lungtransplanterade patienter utvecklar CLAD någon gång under de första fem åren.

I studie 3, undersökte vi effekten av ischemisk tid (IT) vid lungtransplantation (ischemisk tid är den tid som donator lungor utan cirkulation under transport mellan donator och recipient). Vi har kommit till resultat att varje 2-timmarsökning av IT motsvarar en ökad dödlighet på upp till 24 % inom 5 år och IT ger en nyckelroll för att förbättra LTx-resultatet.

I studie nr. 4 utforskade vi rollen som biomarkörer i plasma har hos patienter i den största undergruppen av CLAD, det vill säga patienter med BOS. Plasma från lungtranplanterade patienter med olika stadier av BOS analyserades för uttryck av biomarkörer. Markören corticotropine releasing hormone (CRH) hittades och olika stort uttryck av denna biomarkör kunde relaterades till olika stadier av BOS. CRH identifierades således som en ny potentiell markör för diagnostik av kronisk rejektion efter lungtransplantation.

Sammanfattningsvis så är det övergripande syftet med min avhandling att förbättra den långsiktiga överlevnaden hos patienter som genomgår lungtransplantation, samt att utforska möjligheterna till utökning av donatorpoolen. Detta kommer att vara av stor betydelse för patienterna som fortfarande väntar på sina nya lungor och alla de som kommer att komma efter dem.

3 Abbreviations

AECC	American–European Consensus Conference
ALI	Acute lung injury
Anti-HLA	Antihuman leukocyte antigen
ARDS	Acute respiratory distress syndrome
BALF	Bronchoalveolar lavage fluid
BLT	Bilateral lung transplant
BOS	Bronchiolitis obliterans syndrome
BPM	Breaths per minute
CAD	Coronary artery disease
cDCD	Controlled donation after circulatory death
CF	Cystic fibrosis
CI	Confidence interval
CLAD	Chronic lung allograft dysfunction
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CRH	Corticotropin-releasing hormone.
CT	Computerised tomography
CVP	Central venous pressure
DBD	Donation after brain death
DCD	Donation after circulatory death
DO_2	O_2 delivery
EBV	Epstein-Barr virus
EDC	Extended donor criteria

ELISA	Enzyme-linked immunosorbent assay
EVLP	*Ex vivo* lung perfusion
FCER2	Fc Epsilon Receptor II
FEF	Forced expiratory flow
FEV_1	Forced expiratory volume 1
FIO_2	Fraction of inspired oxygen
GMP	Good manufacturing practices
Hc	Haematocrit
HGF	Hepatocyte growth factor
HR	Hazard ratio
I/R	Ischaemia/reperfusion
ICU	Intensive care unit
IIP	Idiopathic interstitial pneumonia
IL	Interleukin
ILD	Interstitial lung disease
IPAH	Idiopathic pulmonary arterial hypertension
IRI	Ischaemia–reperfusion injury
ISHLT	International Society for Heart and Lung Transplantation
IT	Allograft ischaemic time
LA	Left atrium
LAM	Lymphangio-leiomyomatosis
LPS	Lipopolysaccharide
LTx	Lung transplantation
LVEDP	Left ventricular end diastolic pressure
MAPC	Multipotent adult progenitor cell
mLAP	Mean left atrial pressure
MMP-9	Matrix metalloproteinase 9
mPAP	Mean pulmonary arterial pressure
MSCs	Mesenchymal stromal cells

N_2	Nitrogen
OCS	Organ Care System
OR	Operating room
P/F ratio	Partial pressure of arterial oxygen/Fraction of inspired oxygen
PaO_2	Partial pressure of arterial oxygen
PAP	Pulmonary artery pressure
PAWP	Pulmonary artery wedge pressure
PBS	Phosphate-buffered saline
PCT	Procalcitonin
PEA	Proximity extension assay
PEEP	Positive end-expiratory pressure
PGD	Primary graft dysfunction
PGF	Primary graft failure
PLTRE	Post–lung transplantation reperfusion oedema
PVR	Pulmonary vascular resistance
RAS	Restrictive allograft syndrome
RBC	Red blood cells
RND	Resistance-nodulation-division
ROS	Reactive oxygen species
RR	Respiratory rate
SD	Standard deviation
SEM	Standard error of the mean
SLT	Single lung transplant
TNF	Tumour necrosis factor
TTX	Thoracic Organ Transplant Registry
TUNEL	TdT-mediated dUTP-biotin nick end labelling
uDCD	Uncontrolled donation after circulatory death
VO_2	Maximal oxygen consumption
XPS	XVIVO Perfusion System

4 Introduction
The respiratory system

4.1 Anatomy and physiology

The human respiratory tract can be divided into the upper respiratory tract (extrathoracic organ – nose, mouth, pharynx, and larynx) and the lower respiratory tract (intrathoracic organ – trachea, bronchi, bronchioles, alveolar duct, and alveoli). (*Figure 1*).

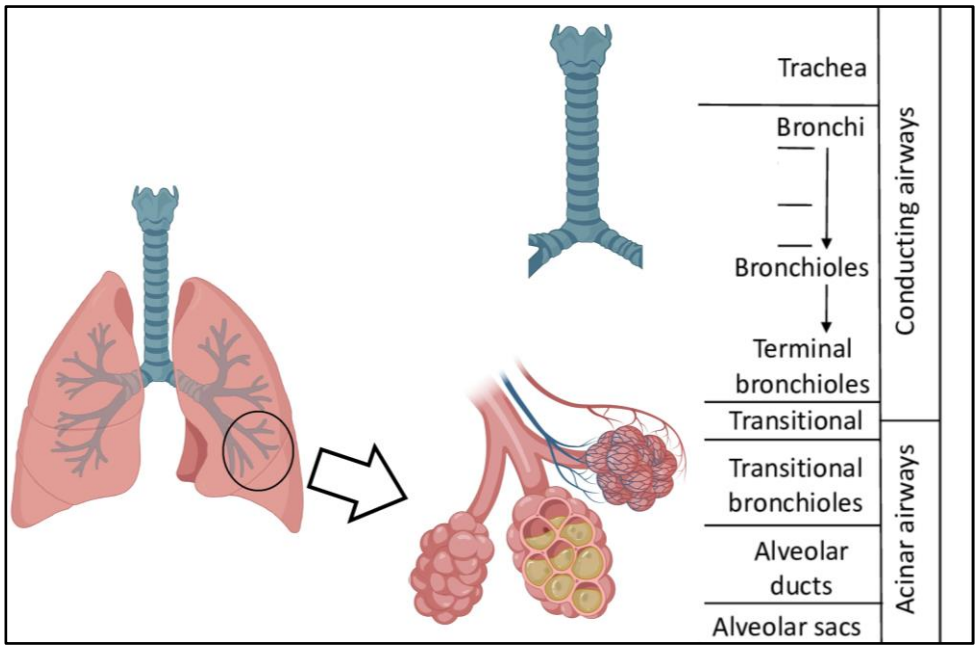


Fig. 1.Respiretory truck anatomy
Created with BioRender.com

Alveoli are the major sites of gas exchange. Humans have about 480 million alveoli making 50 to 75 m^2 of surface area for the gas exchange process[1].

The alveolar wall comprises a simple epithelial lining membrane consisting mostly of type I cells or pneumocytes I which facilitate gas exchange and maintain the fluid balance within the alveoli. In addition, there are a small number of another type of cells called type II, or pneumocytes II, which secrete surfactant which is a liquid that covers the inner surface of the alveoli. Gas exchange occurs in the alveoli where oxygen (O_2) is exchanged with carbon dioxide (CO_2) across the air-blood barrier (*Figure 2*).

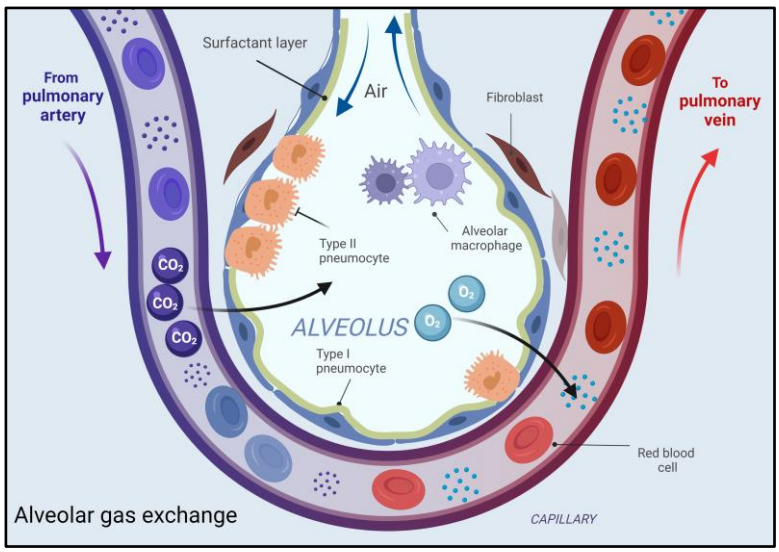


Fig. 2. The cross section of an alveolus with capillaries shown. Part of the cross section magnified to show diffusion of oxygen gas and carbon dioxide through type I cells and capillary cells. *Created with BioRender.com*

The chest wall or thoracic cavity consists of 12 paired ribs attached anteriorly to the sternum via costal cartilages and fused posteriorly together with the 12 thoracic vertebra. The thoracic cavity protects the vital thoracic organs from external trauma and supports breathing and stabilises the shoulder girdle and upper arms during movement. The diaphragm operates like a *blacksmith's bellows*, drawing air into the lungs and then pushing it out at regular intervals.

The primary function of the respiratory system is to deliver O_2 into the cells, which is necessary for their activities and removal of CO_2. Inhaled O_2 enters the lungs and reaches the alveoli. The alveoli wall is just one cell thick, which facilitates the gas

exchange process. O_2 passes quickly through this air-blood barrier into the blood in the capillaries.

Gas exchange is the main vital function of the lungs; the lungs also have another particularly important function, i.e. regulating the pH balance in the body which is called the acid-base balance, thereby changing the level of CO_2 through a change in the ventilation pattern[2].

4.2 Pulmonary circulation

The main function of the pulmonary circulation is to participate in the gas exchange process at the air-blood barrier. The pulmonary artery consists of a thin, elastic vessel with incomplete circumferential layers of smooth muscle in the media, unlike the systemic circulation vessels, which have a complete circumferential layer of smooth muscle cells in the media of the arterioles, which regulates resistance[3].

4.3 Pulmonary vascular resistance (PVR)

Pulmonary vascular resistance (PVR) reflects the resistance against blood flow from the pulmonary artery to the left atrium. The pressure drop from the pulmonary arteries to the left atrium is approximately 10 mmHg compared to a 100-mmHg pressure gradient in the systemic circulation. If the pressure in the pulmonary vasculature is high, the right ventricle must work harder to move the blood forward to the pulmonary valve. Over time, this may cause dilatation of the right ventricle, and require additional volume to maintain the left ventricle preload.

The standard formula for calculating PVR is as follows:

$$PVR = \frac{\text{mPAP} - \text{mLAP}}{\text{Cardiac Output}}$$

**mPAP is the mean pulmonary arterial pressure*

**mLAP is the mean left atrial pressure or PAWP is the mean pulmonary artery wedge pressure*

The method of measuring cardiac output in our Papers I and V is transpulmonary thermodilution technique via a pulmonary artery catheter. Sufficient tissue oxygenation is dependent on three factors: O_2 delivery (DO_2), O_2 binding in the blood and the ability of the cells to take up and utilise the O_2 delivered which is called maximal O_2 consumption (VO_2).

The main cause of tissue hypoxia is an imbalance between DO_2 and VO_2. Insufficient DO_2 can either be the result of pulmonary causes, such as inadequate pulmonary function, or extra-pulmonary reasons, such as poor cardiac function or transportation capacity disturbances (e.g. anaemia). Invasive haemodynamic cardiopulmonary monitoring is necessary for the majority of patients admitted to the intensive care unit (ICU) or in the operating room (OR) for assessing patients who are at risk of hypoxia and for choosing appropriate management.

4.4 Cardiopulmonary circulation

The pulmonary circulation is a high-flow and low-pressure circuit which includes a huge plexus of arterioles and veins between the heart and lungs. The cardiopulmonary circulation is divided into two separate systems (right and left circulation system).

4.4.1 Right heart

The right heart receives deoxygenated blood from the systemic circulation via the superior and inferior vena cava into the right atrium then into the right ventricle which is a low-pressure pump with a thin muscular wall. The deoxygenated blood is ejected into the pulmonary artery which arises from the right ventricle and runs a course of only a few centimetres before dividing into the right and left main branches then numerous subsequent branches to form an extensive network of small arteries, arterioles and capillaries.

O_2 and CO_2 pass over the blood-air membrane during the gas exchange process. The output from the right ventricle is assessable and calculable with a pulmonary artery catheter connected to a pressure transducer[4] (*Figure 3*).

4.4.2 Left heart

The left heart receives oxygenated blood from the pulmonary circulation to the left atrium via four pulmonary veins, then the thick ventricular muscular forces oxygenated blood through the aortic valve to be distributed into the systemic circulation. The red blood cells (RBC) deliver O_2 to the tissues as they pass through the small capillaries and simultaneously bind CO_2 that is produced by the cells. The output from the left ventricle is assessable and calculable with an arterial line connected to a pressure transducer.

4.5 Pulmonary artery catheter

Dr H.J.C Swan and *Dr William Ganz* and colleagues invented pulmonary artery catheterisation for haemodynamic monitoring and, in the 1970s, they introduced the balloon-tipped, flow-directed, pulmonary artery catheter into clinical practice[5].
The catheter is introduced through a large vein — often the internal jugular, subclavian, or femoral veins — and located into the right atrium where central venous pressure (CVP) is assessable and the balloon is inflated and then moved further forward into the right ventricle where the right ventricular pressure can be measured. After this it is then moved forward again into the pulmonary artery where systolic, diastolic, and mean pulmonary pressures can be measured continuously. The cardiac output can be monitored continuously when the balloon is deflated.

A Swan–Ganz catheter can used to monitor the left heart function when the balloon remains inflated by advancing it further into the pulmonary circulation until a pulmonary artery wedge pressure (PAWP) is achieved. PAWP reflects the pressure in the left atrium and when the mitral valve opens in diastole, the left ventricular end diastolic pressure (LVEDP) can measured[4] (*Figure 3*).

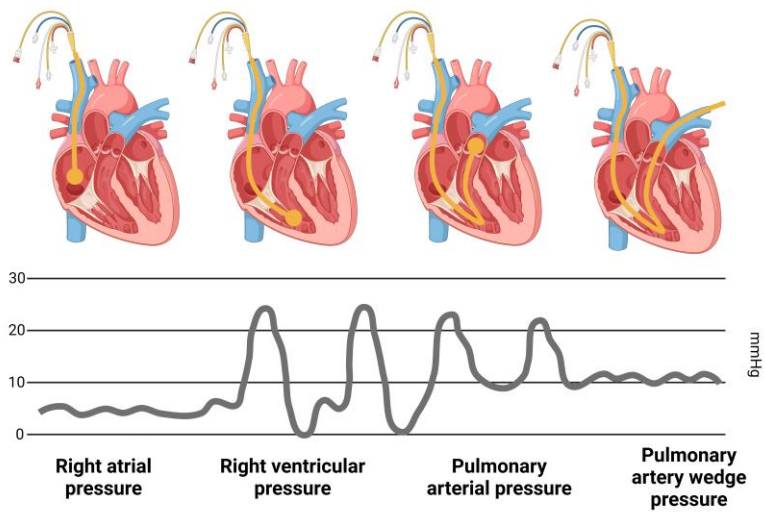


Fig. 3. Pressure curves with normal values with a Swan-Ganz catheter.
From left: right atrial pressure 2-6 mmHg, right ventricular pressure systolic 15-25 mmHg and diastolic 0-8 mmHg, pulmonary arterial pressure systolic 15-25 mmHg and diastolic 8-15 mmHg, pulmonary artery wedge pressure 8-12 mmHg. *Created with BioRender.com*

5 Historical perspectives of lung transplantation

Pioneers of thoracic transplantation

Since the introduction of heart-lung transplantation in the 1960s, thoracic transplantation has improved over time and become an established and effective therapeutic option in end-stage pulmonary disease, both in terms of survival and quality of life[6].

In June 1963, *James Hardy* became the surgeon who performed the first human lung transplant in the world with the recipient surviving for 18 days[7].

A few days later, *George Magovern* and *Adolph Yates* performed the second human lung transplant with the recipient surviving for 26 days[8].

The first "successful" lung transplant, in which the recipient survived for 10 months, was reported by *Fritz Derom* in 1971[9].

Ten years later, *Bruce Reitz* and his team performed the first successful combined (*en bloc*) heart-lung transplantation[10].

Seven years later, *Alexander Patterson* performed the first successful double lung transplant[11].

Stig Steen, at the University Hospital of Lund, first described the clinical application of *ex vivo* lung perfusion (EVLP) in 2001[12].

As experience with lung and heart transplantation procedures has developed over the last six decades, lung transplantation has become a gold standard therapy in the management of end-stage pulmonary disease.

6 Surgical techniques and approaches

The surgical approaches to lung transplantation have been excellently standardised for the past three decades into four main different surgical techniques: single-lung transplantation, bilateral sequential transplantation, heart-lung transplantation, and finally, transplantation of lobes from living donors. Lung transplantation is usually performed without cardiopulmonary bypass (ECC) or intraoperative extracorporeal membrane oxygenation (ECMO) support, but in many high-volume centres it is a routine practice that uses ECMO during the procedure[13, 14].

The traditional incision for bilateral sequential lung transplantation is Clamshell access[15-17], but sternotomy is performed routinely nowadays as a general access to heart-lung transplantation in many high-volume centres[18].

Single-lung transplantation has been the most commonly used surgical approach because it is a relatively quick and easy approach and one donor can be used for two recipients[19]. This procedure has been used successfully in patients with all types of lung disease except cystic fibrosis (CF) and bronchiectasis. In addition, it is not recommended for patients with primary pulmonary hypertension[20].

Bilateral sequential transplantation, or sometimes called double or sequential single lung transplant, involves the sequential performance of two single-lung transplantations during one operating period. It is usually performed without cardiopulmonary bypass by ventilating the contralateral lung during each implantation. This approach replaced the previous technique of *en bloc* double-lung replacement[21].

Transplantation of lobes from living donors

This technique has been developed recently and has been performed exclusively in patients with CF. The technique involves the removal of the right and left lower lobes from two healthy donors. These are then implanted into a recipient after bilateral pneumonectomies using cardiopulmonary bypass[22] (*Figure 4*).

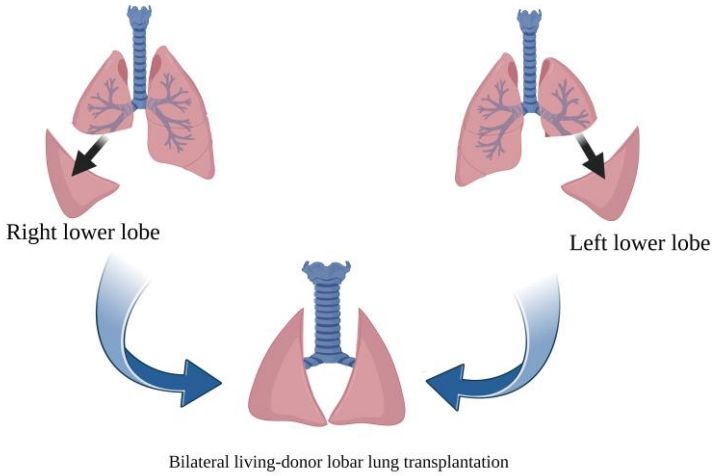


Fig. 4. Bilateral living-donor lobar lung transplantation. Right and left lower lobes from two healthy donors are implanted in a recipient in place of whole right and left lungs, respectively.
Created with BioRender.com

6.1 Post-lung transplantation

6.1.1 Complications

6.1.1.1 Primary graft failure or primary graft dysfunction

Primary graft dysfunction (PGD) is still the major cause of early mortality and morbidity after lung transplantation, occurring in between 10% to 30% of lung transplant recipients. Approximately 15% of recently transplanted allograft recipients developed mild transient pulmonary oedema and a form of acute respiratory distress syndrome termed primary graft failure or dysfunction which is due to *ischaemia-reperfusion injury* (IRI). The appearance of widespread infiltrates on chest radiographs and progressive hypoxaemia within 72 hours' post-transplantation are the main clinical manifestations of PGD, but first other causes for graft failure should be excluded, such as pneumonia, occlusion of the venous anastomosis, aspiration, and others[23-28].

(*PGD is described in detail in chapter "Allograft dysfunction"*)

6.1.1.2 Airway complications

The improvement of the surgical techniques and successful management of the postoperative period have led to significantly reduced airway complications which were the major cause of morbidity and mortality. Complete dehiscence of bronchial anastomosis is the fatal airway complication that requires immediate surgical correction or re-transplantation, while partial dehiscence can be managed conservatively. Anastomotic stenosis is also the common airway complication that typically occurs several weeks or months after transplantation with the main clinical symptoms including focal wheezing and recurrent lower respiratory tract infection. It requires immediate correction with stent placement by broncho-scopy[29-32].

6.1.1.3 Infection

The incidence of post-lung transplantation infection is higher than in recipients of other organs and is most probably related to the exposure of the allograft to the external environment.

Bacterial pneumonia is a common infection in the early period after transplantation; the incidence is highest in the first month. The most common causative organisms are *Gram-negative bacilli*, such as *Klebsiella* organisms, *Pseudomonas* and *Enterobacter*. Gram-positive organisms responsible for infection include *Staphylococcus aureus.*

Fungal infections usually occur between 10 and 60 days after transplantation, most commonly caused by *Candida* and *Aspergillus* organisms.

Cytomegalovirus was the most common viral agent, typically occurring in the second month after transplantation. *Pneumocystis carinii* infections were common 4 to 6 months after transplantation. Mortality due to infection has been associated with 40% of all deaths[33] (*Figure 5).*

6.1.2 Follow-up

There is no standard international post-lung transplantation follow-up protocol, but in general, all recipients undergo extensive and careful clinical, radiological, and pulmonary function monitoring and follow-up at consistent intervals of 3, 6, and 12 months, then annually. The regimen follow-up includes complete spirometry, Six Minute Walk Test (6MWT), blood tests such as glomerular filtration rate and serology/virology, high-resolution computed tomography and transbronchial biopsies and bronchoalveolar lavage.

6.1.3 Causes of death

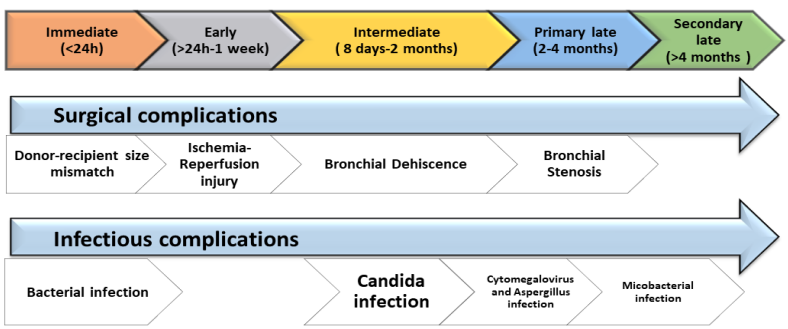
The major cause of 30 days' post-transplantation mortality is a form of acute respiratory distress syndrome or diffuse alveolar damage called *PGD/Acute rejection* (AR) while long-term mortality is caused by *chronic lung allograft dysfunction* (CLAD)[34, 35].

For a long time, bronchiolitis obliterans syndrome (BOS) was the only manifestation of chronic lung dysfunction. Terms such as "chronic rejection" and "BOS" were generally used, but nowadays chronic rejection is defined clinically as a cause of CLAD which includes all variants of pulmonary chronic dysfunction[36, 37]. The incidence of CLAD is unexpected within the first year after lung transplantation, but the risk rises rapidly with an incidence as high as 40%–80% up to 5 years after the procedure[34, 37].

(*PGD and CLAD are described in detail in `chapter "Allograft dysfunction")*

6.1.4 Survival

Post-transplantation survival rates have improved only moderately over the last 10 years, despite improvements in surgical technique and careful management of the recipient by the postoperative intensive care team. These rates are considered low rates compared to those for heart and liver transplantation for which 5-year survival is now approximately 70%[38]. The Registry of the ISHLT (2017) has reported 1-year, 3-year, 5-year and 10-year survival rates after lung transplantation as being 80%, 65%, 54% and 32%, respectively, with a median survival of 5.8 years among the adult patients who underwent primary lung transplant between January 1990 and June 2014[39].



Radiological features of ischemia-reperfusion injury after lung transplantation.
S. Fraia, E. Faccioli, R. Polverosi, M. Loy, E. Quaia, F. Rea, C. Giraudo; Padova/IT
DOI: 10.26044/ecr2020/C-07745

Fig. 5. Post-lung transplant complications.

7 Recipient selection criteria

Lung transplantation remains the only lifesaving option for individuals with end-stage lung disease, as well as improving the patient's quality of life. Survival after lung transplantation is dependent on recipient and donor selection criteria.

In recent years, advances in surgical techniques, improvement of graft preservation and advancements in immunosuppressive therapy have improved both short- and long-term survival rates since the 1980s and 1990s. Candidates for lung transplantation are patients who have been diagnosed with chronic irreversible pulmonary disease, that is unresponsive to other medical and/or surgical treatment and usually symptomatic during normal daily living activities and who have a limited life expectancy of less than 18 months and who are dependent on supplemental oxygen.

Recipients are selected according to the guidelines by the agreement report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation (ISHLT)[40-42], then reviewed by a multidisciplinary team before they are accepted and listed for transplantation[43].

In the most recent official lung and heart-lung transplant registry report released by the ISHLT and the International Thoracic Organ Transplant Registry (TTX), substantial data were recorded regarding transplant procedures, donor and recipient characteristics and outcomes from a global community of transplant centres. The Registry includes data on almost 70,000 adult lung transplant procedures since its inception[44].

7.1.1 Diagnosis criteria of recipients

Lung transplantation should be considered for adults with chronic, end-stage lung disease who meet all the following general criteria[40]:

I. High (>50%) risk of death from lung disease within 2 years if lung transplantation is not performed.
II. High (>80%) likelihood of 5-year post-transplant survival from a general medical perspective, provided that there is adequate graft function.

Generally, lung transplantation candidates should have a chronic, progressive pulmonary disease, such as chronic obstructive pulmonary disease (COPD), alpha1 antitrypsin deficiency (AAT1), interstitial lung disease (ILD), cystic fibrosis (CF), non-CF bronchiectasis, pulmonary hypertension (PH), retransplantation, idiopathic interstitial pneumonia (IIP) and some less common indications, such as sarcoidosis, lymphangio-leiomyomatosis (LAM), BOS, etc[45, 46].

7.1.2 Age of recipients

The maximum age limit for lung transplantation candidates remains a controversial subject. Lung transplantation as a surgery is complicated and carries a risk of perioperative morbidity and mortality. Recipient age therefore greatly affects the clinical outcome. There is strong evidence from the literature that older patients have a worse outcome[47, 48].

In the 2006 and 2014 guidelines, age greater than 65 years was considered to be a relative contraindication to lung transplantation. However, the number of lung transplant recipients aged ≥ 60 years has increased worldwide over the past decade from about 20% in 2000 to >40% in 2012[49].

In the USA, candidates older than 65 years of age now comprise more than 30% of the waiting list and are the age group with the highest transplant rate[47, 50].

The current guidelines no longer recommend an upper age limit for lung transplantation but, in most lung transplant centres, adults over the age of 75 years are usually unlikely to be considered as candidates for a lung transplant[51-53].

7.1.3 Coronary artery disease (CAD) and lung transplantation

Multiple retrospective studies over the past 5 years have shown that patients with mild-to-moderate CAD or those who have undergone revascularisation for CAD may not have worse survival compared to patients without CAD[54, 55].

In view of the results of these studies, CAD should not be considered to be an absolute contraindication, but such patients have been highly selected and consultation with a cardiologist is mandatory for pre-transplant assessment and evaluation of other risk factors that may increase the risk for adverse post-lung transplant outcomes, such as advanced age, low glomerular filtration rate, reduced left ventricular ejection fraction, peripheral vascular disease, very high or very low body mass index (BMI), hypoalbuminaemia, poorly controlled diabetes, re-transplantation, previous pleurodesis and others[40].

8 Addressing the shortage of donor lungs for transplantation

8.1 Introduction

Lung transplantation is still limited by the scarcity of appropriate donor lungs for transplantation which has led to an increasing gap between the number of suitable lung donors and the number of patients on waiting lists. Over the last decade, there has been an increase in the number of listed recipients requiring hospital admission, often for mechanical ventilator support and/or ECMO support prior to transplant. Currently only approximately 15% to 20% of potential donor lungs are acceptable for transplantation[56, 57].

The reason for this low acceptability rate is multifactorial, and includes challenges such as *neurogenic pulmonary oedema (NPE),* which is defined as acute respiratory distress caused by severe sympathetic discharge from an acute central nervous system accident leading to the clinical picture of a large accumulation of extra-vascular pulmonary fluid. Prognosis of NPE is generally poor due to the severity of the underlying brain injury, with estimated mortality rates of between 60 and 100%[58].

Another common reason is *aspiration of gastric contents* which carries significant morbidity for hospitalised patients. The severity of pulmonary injury depends on the presence of particulate matter, volume, and pH of aspirate. Aspiration of gastric contents leads to an intense parenchymal inflammatory reaction. The first phase between 1-2 hours after aspiration is caused by a direct chemical effect of low pH on the alveolar capillary lining cells. The second phase between 4-6 hours is associated with infiltration of neutrophils into alveoli causing a histological picture of acute inflammation[59].

Additionally, there are several other reasons for lung injuries, such as *ventilator-associated injury, pulmonary thrombosis/embolism,* or injury to the lung itself which may occur before or after brain death.

8.2 Donor selection

The accepted donor's lungs are considered when the following criteria are matched: age <55 years, clear chest radiograph, arterial oxygen pressure (PaO_2) > 300 mmHg on fraction of inspired oxygen (FiO_2) = 1.0 and positive end expiratory pressure (PEEP) = 5 cm H_2O, ABO compatibility, adequate size match, smoking history <20 pack-years, absence of chest trauma, no evidence of aspiration, absence of organisms in sputum and no purulent secretions on bronchoscopy. These criteria were proposed in 1993 by Sundaresan *et al.*[60] but a comprehensive survey published by the ISHLT in 2003 approved that these existing standard lung donor criteria had been established in an earlier period of lung transplantation based mainly on opinions and individual experiences rather than on than solid medical evidence[60-62].

During the past decade, most transplantation centres accept only 15–20% of donors due to these strict and inflexible standard criteria[53].

What is required are extended criteria and strategies to increase the donor pool.

8.3 Extended donor criteria (EDC)

EDC means using donor lungs that do not meet the standard criteria for transplantation, such as the acceptance of advanced donor age, minor chest radiograph abnormality, lower PaO_2, types of malignancy, certain forms of donor treatable infection, chest trauma, and smoking history >20 pack years[61, 63].

Reviews suggests that a history of smoking or asthma should not be considered to be absolute contraindications if radiographic imaging and P/F ratios are >300[64, 65].

However, matching virology such as Epstein-Barr virus (EBV) or hepatitis C (antigen positive or negative) managed with novel hepatitis C treatment, does not impact upon clinical outcomes[66].

8.3.1 *Ex vivo* lung perfusion (EVLP)

Optimisation of unsuitable donor lungs using *ex vivo* lung perfusion (EVLP) can increase the donor pool in two ways. Firstly, EVLP aids in reconditioning and improves lung physiology through optimisation of arterial partial pressure prior to transplantation. Secondly, EVLP remove the harmful effects of cold ischaemia and transport of donor lungs through a mobile device called Organ Care System (OCS) before the donor organs reach the critical ischaemic time that would affect long-term survival after lung transplantation[67-71].

(*EVLP is described in detail in chapter "Ex vivo lung perfusion"*)

8.3.2 Donation after circulatory death (DCD)

DCD is one of non-traditional organ donation methods that is currently performed as a way of expanding the donor pool. *Ehrsam et al.* estimated about 50% might potentially increase the total donor pool and would significantly decrease the mortality rate on the waiting list[72]. A DCD lung donor is an appropriate non-brain death person who has a fatal terminal cardiac disease, neurological but non-brain death disease or, in rare cases, respiratory disease such as an acute central lung embolism leading to a circulatory arrest on condition that resuscitation is not to be attempted or continued. The time period that legally constitutes 'brain dead' is called the Standoff period which is between 2 and 20 minutes, depending on jurisdiction[73].

9 The role of cytokines in lung transplantation

Cytokines are low molecular weight soluble proteins produced by different immune cells and other cells. Cytokines are recognised as key players in the development of pro- and anti-inflammatory responses. They might be used for early diagnosis of injurious inflammatory events, such as PGD and acute lung injuries[74].

Cytokines associated with PGD and CLAD

PGD is a form of ischaemia/reperfusion lung injury (I/R) that leads to early post-transplantation morbidity and mortality. CLAD is the leading cause of late mortality. There are different phenotypes of CLAD which have been described, such as BOS and restrictive allograft syndrome (RAS)[36, 75, 76].

Several clinical and preclinical studies have been employed to detect several cytokines associated with PGD and CLAD, such as the following[77, 78].

Interleukin-6 (IL-6)
IL-6 is one of the cytokines produced by alveolar macrophages, lung parenchyma, and other cells in response to injury and infection.

Wen and associates reported that pulmonary complications following liver transplantation were associated with increased serum concentrations of tumour necrosis factor-alpha (TNFα), IL-6 and IL-8, suggesting that they occur secondary to pulmonary injury after hepatic I/R[79]. However, *Pham and colleagues* found that early elevations in IL-6 correlated with later allograft dysfunction[80-83].

An animal model of lung transplantation showed that IL-6 is involved in T-cell stimulation and the generation of T-regulatory cells; both cells play a role in the development of reperfusion injury[84].

Interleukin-8 (IL-8)

Andrew J Fisher and associates published an article which supported the theory that IL-8 contributes to lung injury through increased IL-8 *levels* in both donor bronchoalveolar fluid and allograft tissue associated with primary graft failure as a response to chemotaxis and neutrophil recruitment, and several research groups have published similar results[82, 85, 86].

Interleukin-10 (IL-10)

IL-10 has long been recognised as having broad-spectrum anti-inflammatory activity, which has been confirmed in various models of infection, inflammation, and even in cancer studies. Several experimental models have employed IL-10 to limit reperfusion injury, with promising results[83, 87-89].

Tumour necrosis factor alpha (TNFα)

Welborn and colleagues examined the changes in plasma cytokine concentrations in patients following abdominal and thoracoabdominal aortic aneurysm repair, with and without left atrial femoral bypass. Their study showed that elevations in TNFα and IL-6 were associated with I/R injury which is the main cause of postoperative single or multiorgan dysfunction[90].

Thereafter *Mathur and colleagues* published a study showing that a graded increase in IL-6, IL-8, and IL-10 concentrations occurred pre- and post-allograft perfusion and was also higher in TNFα and IL-10 in primary graft failure (PGF) patients[82].

10 Impact of cytokine adsorption on lung transplantation outcome

Multiple preclinical and clinical trials have explored the use of adsorbers in severe sepsis cases. In 2019, *Hawchar and colleagues* published details of the first randomised, controlled pilot study to investigate the effects of early extracorporeal cytokine adsorption treatment in septic shock without renal replacement therapy. The study presented the extracorporeal cytokine adsorption method as a safe technique with significant effects on norepinephrine requirements, and procalcitonin PCT[91].

Several studies, both preclinical and clinical trials, demonstrated IL-6, IL-8, IL-1βn and TNFα as known cytokines associated with the progression of IRI and acute respiratory distress syndrome (ARDS). Use of the adsorbers is effective in reducing the concentration levels of these cytokines[80, 82, 84, 85, 90, 92, 93].

Furthermore, preclinical studies using animal models of sepsis have demonstrated reductions in various circulating cytokines: by using cytokines, haemoadsorption reduced organ injury, and improved survival[94, 95].

Kellum et al. published a randomised controlled experimental endotoxaemia laboratory study which demonstrated that TNFα, IL-6 and IL-10 were removed rapidly with <50% of the initial concentrations present after 1 hour of circulation through haemoadsorption[96].

Furthermore, other studies have investigated the benefit of using of adsorbers in human orthotopic heart transplantation and in human kidney transplantation[97, 98].

In 2010, *Kakishita et al*. published a porcine study to investigate the change in proinflammatory cytokines of the perfusate during EVLP and to evaluate the effect of adsorbent membrane on the removal of cytokines. This study showed that TNFα and IL-8 levels were significantly lower in the membrane group than in the control group during the EVLP period[99].

Furthermore, two studies from *Iskender et al.* were published utilising an IRI porcine model where lungs were kept in cold ischaemia for 24 hours to then be

placed on 12 hours of EVLP. In their 2017 publication in which a cytokine adsorber was connected to EVLP, they showed improved airway pressures, dynamic compliance, pulmonary oedema and reduced lactate levels as well as a range of diminished cytokines, including IL-1β, IL-6 and TNFα in the treated group compared with the control group[100].

Their 2021 publication utilised the same protocol of IRI and EVLP, except that EVLP time was reduced to 6 hours and the left lung was transplanted and monitored for 4 subsequent hours. The study showed similar previous findings, such as reduced cytokine concentrations in the EVLP perfusate and after transplantation with higher dynamic compliance in the treatment group[101] (*Figure 6*, *Table 1*).

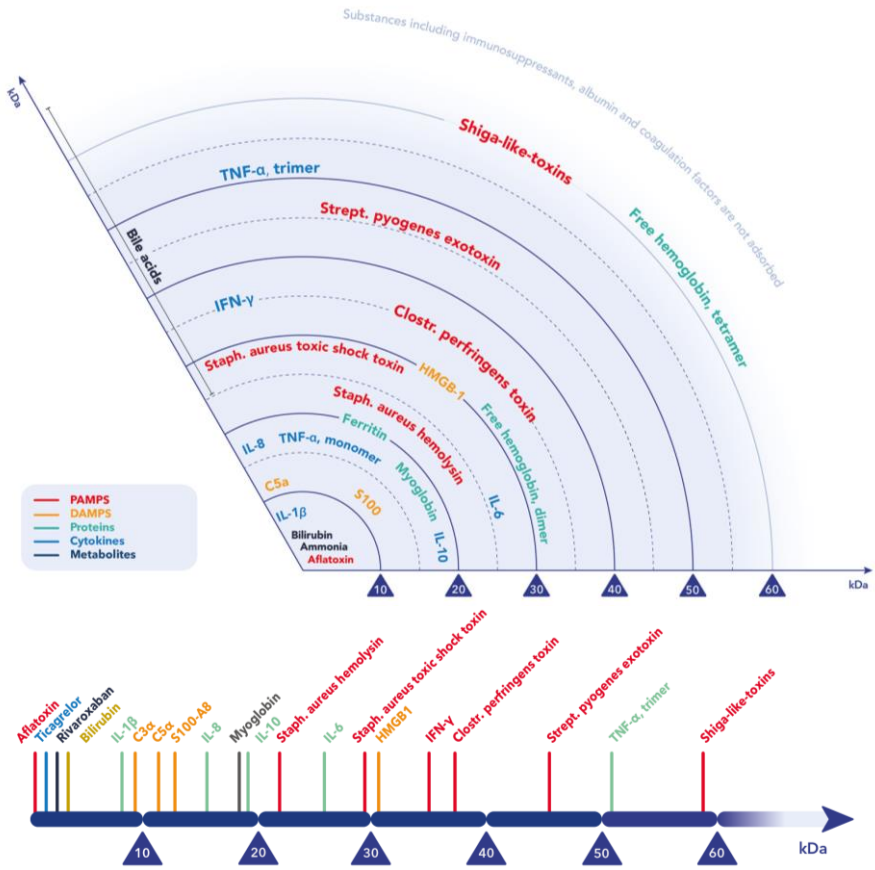


Fig. 6. Adsorption spectrum of the Cytosorb® adsorber as a function of molecule size.

Table 1. Summary of cytokine adsorption/EVLP studies.

Author, Year	Model, Subject Number	Lung injury model	EVLP length	Cytokine filtration type	Oxygenation	Histology	Treatment levels of IL-8	Treatment levels of TNFα
Kakishita *et al.* 2010[99]	Porcine 5–6/group	Not appli-cable	12 h	Lixelle S35	No significant differences between groups	Similar levels of oedema formation between groups.	Significantly lower in treatment group	Significantly lower in treatment group
Iskender *et al.* 2017[100]	Porcine, 5/group	24 h cold ischaemia	12 h	CytoSorb adsorber	Not studied	Lower lung injury scores in treatment group.	Significantly lower plasma levels of all cytokines in treatment group dur-ing EVLP.	Significantly lower in treatment group
Iskender *et al.* 2021[101]	Porcine, 5/group	24 h cold ischaemia	6 h	CytoSorb adsorber	Significantly better venoarterial oxygen pressure gradient in adsorption group after 6 h of EVLP as well as post transplantation	Comparable microscopic lung injury scoring between the groups.	Treatment group after 6 h of EVLP, however no differences found at 8 h post transplantation.	Not studied

Current Status and Future Perspectives on Machine Perfusion: A Treatment Platform to Restore and Regenerate Injured Lungs Using Cell and Cytokine Adsorption Therapy
Anna Niroomand, Gabriel Hirdman, Franziska Olm and Sandra Lindstedt . Accession Number: 35011653 PMCID: PMC8750486 DOI: 10.3390/cells11010091. © 1996-2022 MDPI (Basel, Switzerland)

11 Mesenchymal stromal cells

11.1 Introduction

Mesenchymal stromal cells (mesenchymal stem cells; MSCs) are a spindle-shaped plastic-adherent heterogeneous group of cells that can be isolated from different adult tissues (e.g. bone marrow, adipose tissue, and other tissue sources). These cells are characterised by their ability to differentiate *in vitro*. They were first described in 1974 by *Friedenstein* as haematopoietic supportive cells of bone marrow with a high proliferative ability to differentiate to bone *in vitro* when plated at low density in tissue culture[102]. Several studies have reported advantages and beneficial effects of MSCs in the reduction of inflammation, apoptosis, as well as in the repair and regeneration of lung endothelial and epithelial cells in patients with ARDS[103-106].

11.2 Sourcing of MSCs

Traditionally, MSCs are collected through a bone marrow aspiration procedure; however, many other types of tissue have been identified as alternative sources of MSCs, including adipose tissue, umbilical cord, amniotic fluid, and others[107-110] (*Figure 7*).

11.3MSCs improve acute lung injury (ALI)

MSC therapy has been an extremely attractive approach for experimentation and research in a variety of clinical and preclinical models in the fields of acute lung injury (ALI), septic shock, acute spinal cord injury and others[105].

In the prospective randomised START trial as well as the SafeCell systematic review, MSCs have been confirmed to be a safe and non-harmful method to treat

ARDS[105, 106]. MSCs have become a widespread method within the field of lung transplantation, especially associated with using an EVLP supply that uses a platform to deliver the cells directly to the target organ[103].

In a 2009 application of MSCs during lung perfusion as a treatment method for damaged intrabronchial endotoxin-induced human lobes, the study showed that the lung endothelial permeability had been restored in treated damaged lobes relative to untreated ones[103]. Another study of endotoxin-induced ALI in an animal model explored how MSCs can restore lung function following ALI in mice in combination with a specialised proresolving mediator called lipoxin A4 (LXA4), which can be potentially a new therapeutic approach for patients with ARDS[104].

A 2016 study from the Toronto Lung Transplant Programme examined a prolonged cold ischaemia (18 hours) model in which MSCs isolated from human umbilical cords after the first hour of EVLP were administered then continuing with normothermically MSCs *ex vivo* for 12 hours. The method demonstrated an increased concentration of human vascular endothelial growth factor (VEGF) and a decreased concentration of pig IL-8 in lung biopsies and perfusate[111].

In 2017, an experimental study was performed to evaluate the effect of using multipotent adult progenitor cells (MAPCs) in a warm ischaemia lung injury model. MAPCs were distributed in the airways during EVLP. A reduction in pro-inflammatory cytokines and neutrophils in bronchoalveolar lavage of the MAPC cell group was observed and this effect might play an important role in critically modifying the process of PGD after lung transplantation[112].

In 2019, *Nakajima et al.* published their results of a porcine model of 24 hours of cold storage, after which lungs were divided randomly into an MSC group versus a control group. MSCs were delivered directly into the pulmonary artery during EVLP. After 12 hours of EVLP, followed by a 1-hour second cold preservation period, the left lung was then transplanted and re-perfused for 4 hours. The study reported a significant decrease in cell death markers, reduced lung tissue wet-to-dry weight ratio in the MSC group and a significant increase in lung tissue hepatocyte growth factor (HGF) level in the MSC group compared with the control group. Moreover, ALI pathological scores were significantly lower in the MSC group compared to the control group[113] (*Table 2*).

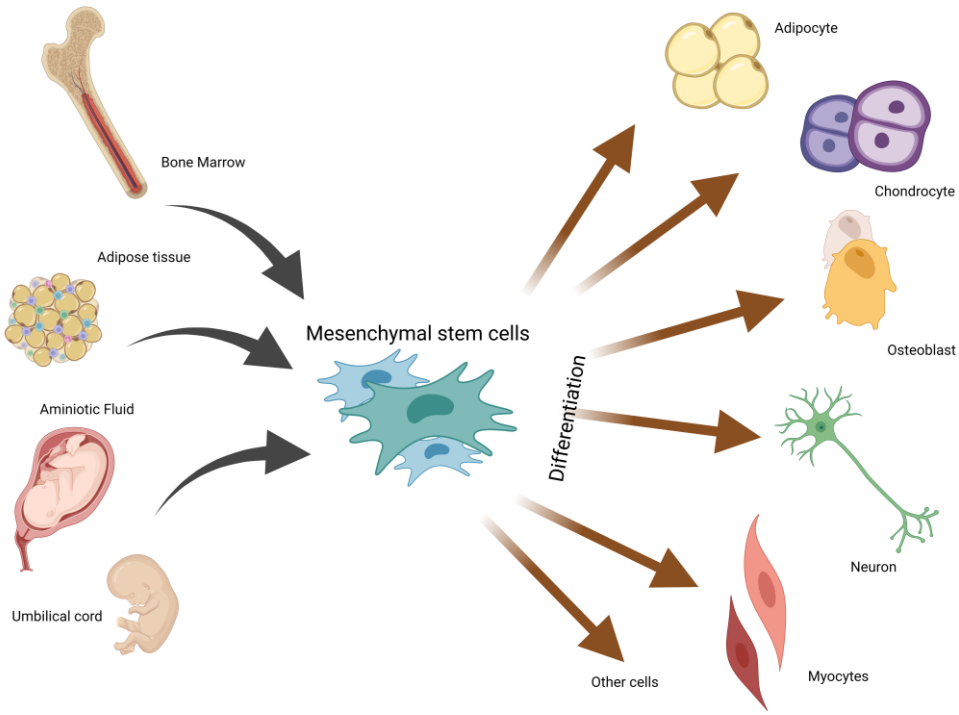


Fig. 7. The sources and characteristics of mesenchymal stem cells.
Created with BioRender.com

Table 2. Studies of MSCs in EVLP.

Author	Year	Model, Subject Number	Experimental Groups	Cell Type	Cell Characteristics	Cell Dose (Total Cells)	Lung Injury Model	EVLP Length	Treatment Levels of IL-8	Treatment Levels of IL-10	Treatment Levels of TNF-α	Pulmonary Function Outcomes
Bone marrow-derived cells												
Martens et al.	2017	Pig, 6/group	MAPCs vs. perfusate (control)	MAPC	Obtained from Athersys/Regenesys (Cleveland, OH, USA) Tested qPCR and flow for negative and positive markers, tube formation assay, CFSE assay	150×10^6	1.5 h warm ischemia, 1 h cold ischemia	6 h	Below detection limit for both groups in BAL	Below detection limit for both groups in BAL	Decreased in BAL	No differences in compliance, oxygenation, or PVR
Fang et al.	2015	Mouse, 12/group	MSCs vs. PBS	MSC	Obtained from Institute for Regenerative Medicine at Texas A&M	5×10^5	In vivo ALI with 5 mg/kg IT LPS	No EVLP	-	-	Decreased in in vivo mice and in coculture of MSCs with ATII cells	Increased 48 h survival rate
McAuley et al.	2014	Human, 3–4/group	MSC vs. perfusate (control)	MSC	Obtained from GMP facility at University of Minnesota, (+) markers: CD73, CD90, CD105 (−) markers: CD14, CD19, CD34, CD45, HLA-DR. Tested for trilineage differentiation	5×10^6	31 +/− 6 h (control) 33 +/− 31 h (MSC) Cold ischemia	4 h	-	-	-	No differences in pulmonary arterial pressures, perfusate oxygenation, AFC restored
Lee et al.	2013	Human, 3–4/group	MSC IV vs. MSC IB vs. normal lung fibroblasts (PromoCell, control)	MSC	Obtained from GMP facility at University of Minnesota; met criteria defined by ISCT	5×10^6	<48 h ischemic time; followed by induction of ALI in EVLP either with 6 mg *E. coli* endotoxin or 10^9 or 10^9 CFU *E. coli* bacteria	6–10 h	Decrease after MSC instillation	In vitro increase in co-culture of MSC with monocytes	In vitro decrease in co-culture of MSC with monocytes	AFC restored
Lee et al.	2009	Human, 3–6/group	MSC vs. conditioned medium vs. normal lung fibroblasts (PromoCell, control)	MSC	Obtained from NIH repository, Tulane Center for Gene Therapy; met criteria defined by ISCT	5×10^6	21 +/− 13 h ischemic time; induced ALI in EVLP with 0.1 mg/kg *E. coli* endotoxin	4 h	MSC not different from injured control	MSC not different from injured control	MSC not different from injured control	AFC restored
Human umbilical cord perivascular cells												
Nykänen et al.	2021	Human 4–5/group	MSCs in one lung vs. perfusate in matched pair lung	MSC modified to produce IL-10	(+) markers: CD73, CD90, CD105, CD10, CD166, CD140b, CD146, MHC I; (−) markers: CD34, CD45, MHC-II; transgene expression of FLAG tag for IL-10 transduction	40×10^6	cold ischemia of 9 h (7.6–12.3) in control, 8.9 h (7.9–11.6) in MSC	12 h	MSC not different from injured control	Increased in tissue and perfusate	-	No difference in PVR, oxygenation, compliance, airway pressure

Table 2. *Cont.*

Author	Year	Model, Subject Number	Experimental Groups	Cell Type	Cell Characteristics	Cell Dose (Total Cells)	Lung Injury Model	EVLP Length	Treatment Levels of IL-8	Treatment Levels of IL-10	Treatment Levels of TNF-α	Pulmonary Function Outcomes
Pacienza et al.	2019	Rat 8–10/group	MSCs vs. vehicle control of Krebs-Henseleit solution	MSC	Obtained from Laboratory of Gene Therapy at Universidad Austral, met ISCT guidelines, (+) markers: CD44, CD90, CD105 (−) markers: CD11b, CD34, CD45	1×10^6	2 h warm ischemia, 90 min cold ischemia	1 h	-	-	-	Compliance decreased by less from baseline in MSC group
Nakajima et al.	2019	Pig, 6/group	MSCs vs. perfusate (control)	MSC	Obtained from Tissue Regeneration Therapeutics, (+) marker: CD73	5×10^6/kg	24 h cold ischemia	12 h	MSC not different from control in EVLP or post-transplant	-	MSC not different from control in EVLP, decreased post-transplant	Peak airway pressure reduced in EVLP, no change in oxygenation, PVR, compliance in EVLP or post-transplant
Stone et al.	2017	Mouse 6–8/group	MSCs vs. EVs vs. Steen Solution vs. Krebs Henseleit buffer	MSCs	(+) markers: CD73, CD90, CD105, CD44 (−) markers: CD45, CD34, CD11b, CD19, HLA-DR Tested for trilineage differentiation	1×10^6 before ischemia, 3×10^6 in EVLP	In vivo 1 h hilar occlusion followed by 2 h reperfusion Or 1 h warm ischemia, 1 h cold ischemia followed by EVLP	1 h	-	Increased in in vivo model	Decreased in in vivo model	Increasing compliance, decreased PA pressure in both in vivo and EVLP models
Mordant et al.	2016	Pig, 3–5/group	IB MSC vs. IV MSC vs. no cells	MSC	Obtained from Tissue Regeneration Therapeutics, (+) marker: CD73	IB: 50×10^6 IV: 50×10^6 150×10^6 300×10^6	18 h cold ischemia	12 h	Decreased in IV MSC	IV MSC not different from control	-	No change in PVR in IV MSC, transient increase in IB, Increased oxygenation, compliance with 150×106 IV dose.
La Francesca et al.	2014	Human, 4	MAPC or sterile saline (control)	MAPC	(+) markers: CD49c, CD90 (−) markers: MHC class II, CD45	1×10^7	8 h cold ischemia	4 h	-	No significant difference in tissue or BAL	-	Reduced injury on histology scoring, reduced neutrophils and eosinophils

AFC—alveolar fluid clearance; ALI—acute lung injury; ATII—alveolar type II cells; BAL—bronchoalveolar lavage; EVLP—ex vivo lung perfusion; IB—intrabronchial; IT—intratracheal; IV—intravenous; LPS—lipopolysaccharide from *E. coli*; MSC—mesenchymal stromal (stem) cells; MAPC—multipotent adult progenitor cells; PBS—phosphate buffered saline. International Society of Cellular Therapy (ISCT) Criteria: (+) markers: CD105, CD73, CD90; (−) markers: CD45, CD34, CD14 or CD11b, CD79α or CD19, HLA-DR; cells must show trilineage differentiation.

Current Status and Future Perspectives on Machine Perfusion: A Treatment Platform to Restore and Regenerate Injured Lungs Using Cell and Cytokine Adsorption Therapy
Anna Niroomand, Gabriel Hirdman, Franziska Olm and Sandra Lindstedt
Accession Number: 35011653 PMCID: PMC8750486 DOI: 10.3390/cells11010091

12 Acute lung injury (ALI)

Acute lung injury (ALI) has most recently been reclassified as mild or moderate ARDS[114]. ARDS is a syndrome of acute respiratory insufficiency that is characterised by tachypnoea, dyspnoea, and progressive arterial hypoxaemia. This acute fatal pulmonary injury always requires ICU admission with endotracheal intubation and positive pressure ventilation.

There are a variety of aetiologies and clinical disorders associated with the development of ALI/ARDS, including sepsis, major trauma, pneumonia, aspiration of gastric contents and inhaled toxic substances[115].

The mortality rate is still very high, ranging from 35% to 65%, despite obvious improvement in the management of ARDS and clinical life support[116, 117].

The life-threatening hypoxaemia among ARDS patients is caused by intra-pulmonary shunt and ventilation-perfusion imbalances. Additionally, deterioration of respiratory system compliance may cause further lung damage with hypercapnia and respiratory acidosis.

Since the first description of ARDS in 1967, the condition has been reviewed extensively and adapted in the last few decades regarding the pathophysiology and essential steps for effective management[118].

12.1 Definition

ARDS was described for the first time in 1967 by *Ashbaugh and colleagues*[118]. However, it remained undefined until 1994 when an international American–European Consensus Conference (AECC) laid the foundations for defining the clinical criteria of ARDS which includes the following: acute onset, bilateral infiltrates on chest radiography, pulmonary-artery wedge pressure ≤18 mmHg or the absence of clinical evidence of left atrial hypertension and $PaO_2/FIO_2 \leq$ 200 mmHg.

The definition criteria for identified ALI are those patients who have bilateral pulmonary infiltrates with $PaO_2/FIO_2 \leq 300$ mmHg[115, 119-121].To define ARDS, the Berlin definition requires all four criteria to be present (*Table 3).*

	Acute Respiratory Distress Syndrome
Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mm Hg < PaO_2/FIO_2 ≤ 300 mm Hg with PEEP or CPAP ≥5 cm H_2O[c]
Moderate	100 mm Hg < PaO_2/FIO_2 ≤ 200 mm Hg with PEEP ≥5 cm H_2O
Severe	PaO_2/FIO_2 ≤ 100 mm Hg with PEEP ≥5 cm H_2O

Abbreviations: CPAP, continuous positive airway pressure; FIO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.
[a] Chest radiograph or computed tomography scan.
[b] If altitude is higher than 1000 m, the correction factor should be calculated as follows: [PaO_2/FIO_2 × (barometric pressure/760)].
[c] This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

Table 3. Berlin definition of acute respiratory distress syndrome.

12.2 Pathogenesis

Disruption of the alveolar-capillary membrane occurs by a variety of mechanisms, either directly through the airway or indirectly via the bloodstream. In the early phase of acute respiratory failure, patients typically develop severe alveolar oedema, with large numbers of inflammatory cells, primarily neutrophils, in the air spaces and interstitium of the lungs. Initially, the oedema fluid has a high concentration of protein, which is characteristic of an increased-permeability pulmonary oedema resulting in a substantial deterioration in gas exchange[120, 122].

Pleural effusions may be noted in 40% of patients with increased-permeability pulmonary oedema[123].

The subacute phase of ARDS occurs approximately from days 5 to 10 after lung injury and primarily involves the interstitium of the lung. Some patients develop an accelerated fibrosing alveolitis. Ultrastructural studies have shown extensive

proliferation of the alveolar type II epithelial cells, apparently in response to injury of the type 1 epithelial cells in the acute phase. There is a pronounced increase in fibroblast and collagen formation in the interstitium.

Lung destruction may occur during the chronic phase of ARDS 10-14 days after the onset of the syndrome. In this chronic phase, patients may have lesser degrees of oxygenation impairment and lesser PEEP requirements; these patients continue to have high dead space and high minute ventilation requirements. Lung compliance may be decreased secondary to pulmonary fibrosis and diminished surfactant synthesis[124] (*Figure 8*).

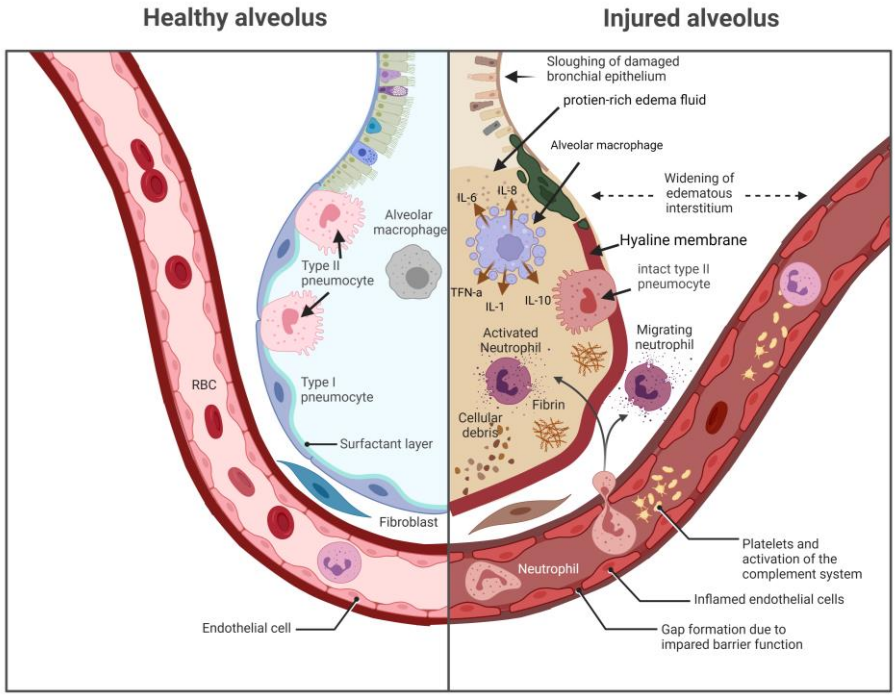


Fig. 8. Alveolar changes in ARDS.
Created with BioRender.com

13 Ischaemia–reperfusion injury in lung transplantation

13.1 Introduction

The condition of IRI is an acute complication following lung transplantation and the main reason for PGD, which is a major cause of mortality and morbidity postoperatively.

In lung transplantation, when the donor lungs are harvested from the donor, the critical ischaemic period begins. The donor lungs are stored in a cold ischaemic state during the transfer time until reperfusion is initiated. The pathophysiological changes begin with an imbalance between the metabolic supply and demand leading to tissue hypoxia causing cellular damage or death. Moreover, the reperfusion of ischaemic lung also stimulates the activation of inflammatory cells resulting in further injury and pulmonary dysfunction[125].

13.2 Pathophysiology

Hypothermic ischaemic storage can lead to acute pathological changes in cells resulting in release of damage-associated molecule patterns (DAMPs) which stimulate the inflammatory response. These molecules can bind to their corresponding receptors, leading to the stimulation of inflammatory cytokine production such as TNFα, and interleukins IL-1β, IL6 and IL12 by immune cells resulting in increased apoptosis which participates in pulmonary dysfunction[126]. Several studies suggest that ischaemia in lung transplantation can result in relevant vascular endothelial changes, such as detachment of endothelial cells, resulting in impaired pulmonary vasodilatation and increased vascular permeability leading to parenchymal oedema and haemorrhage[127, 128]. Soon after the reperfusion period, rapid accumulation of reactive oxygen species (ROS) occurs, which is a type of unstable molecule that affects the DNA of cells, resulting in cell death[129].

14 Allograft dysfunction

14.1 Hyperacute rejection

Hyperacute rejection is a rapidly progressive and fatal graft dysfunction/failure which occurs perioperatively or within the first 24 hours' postoperatively due to an acute reaction between pre-existing antihuman leukocyte antigen (anti-HLA) or anti-ABO antibody in the recipient with the corresponding antigen present in the donor graft causing sudden congestion of the transplant lung and subsequent insufficient graft function. It typically appears in computerised tomography (CT) images as diffuse opacities of the graft.

Hyperacute rejection is a life-threatening condition: plasma replacement, aggressive immunosuppression, and emergency re-transplantation are the only treatments of choice[130-132] (*Table 6*).

14.2 Primary graft dysfunction (PGD)

14.2.1 Definition

PGD is a syndrome of ALI which occurs within the first 72 hours after lung allograft implantation induced by IRI and remains a major cause of early mortality and morbidity. PGD is the most common complication among 10%–25% of patients undergoing lung transplantation.

PGD is characterised by acute pulmonary oedema with diffuse alveolar damage that manifests clinically as progressive hypoxaemia with radiographic pulmonary infiltrates and which develops within 72 hours' post-transplantation[133-136].

14.2.2 Pathophysiology

Principally, PGD shares these pathophysiological aspects with ARDS/IRI. (*Described in chapter "Acute lung injury"*)

14.2.3 PGD manifestation

14.2.3.1 Lung oedema

PGD manifestations increase the permeability of the alveolar-capillary barrier which induces accumulation of fluid in the extravascular spaces of the lung tissue resulting in interstitial pulmonary oedema which subsequently causes impairment of gas exchange at the alveoli level[26, 137, 138].

14.2.3.2 Hypoxaemia

Interstitial pulmonary oedema is responsible for the impairment of gas exchange causing hypoxaemia in PGD. Grading of PGD is based on the severity of hypoxaemia by measuring the PaO_2/FiO_2 ratio[26].

14.2.3.3 Deterioration in pulmonary compliance

Lung compliance reflects the elasticity of the parenchyma. Interstitial pulmonary oedema impairs compliance by interfering with the elasticity of the lung parenchyma[26].

14.2.3.4 Elevation of pulmonary vascular resistance

Multiple physiological and molecular mechanisms are responsible for the elevated PVR; one of these mechanisms is hypoxic vasoconstriction which limits blood flow to the consolidated pulmonary areas. Another mechanism is denervation of the lung grafts which affects vasomotor control and may contribute to the increased PVR[26, 139].

14.2.4 PGD grading

PGD severity, like ARDS severity, is measured by assessing the deterioration in the ratio of PaO_2/FiO_2 or (P/F ratio) associated with the appearance of alveolar infiltrates by chest imaging at four time points starting from reperfusion of the

contralateral lung: T_0 (*within 6 hours of final lung reperfusion*), T_{24} hours, T_{48} hours and T_{72} hours[133] *(Table 4).*

There are secondary causes of graft dysfunction that may progress the grade of PGD, such as postoperative surgical complications, e.g. bronchial, or vascular anastomoses, stenosis, cardiac failure, aspiration, atelectasis, pleural effusion, hemithorax, pneumothorax, and others.

Grade	Bilateral Alveolar Infiltrates on Chest X-ray	PaO_2/FiO_2 Ratio
PGD grade 0	No	>300
PGD grade 1	Yes	>300*
PGD grade 2	Yes	200–300
PGD grade 3	Yes	<200

FiO_2 = fraction of inspired oxygen; PaO_2 = partial pressure of arterial oxygen. PGD = primary graft dysfunction

(nasal cannula oxygen Fio2 < 0.3, or ventilator Fio2 < 0.3)

Table 4. Grading of (PGD) after lung transplantation according to the 2016 definition of the International Society for Heart and Lung Transplantation (ISHLT).

14.2.5 Impact of PGD on outcome

According to the clinical and epidemiological research on PGD, 25–30% of patients develop PGD grade 3 within 72 hours after lung transplantation leading to a dramatically increased early mortality rate (30, 90 days' mortality)[140].

The incidence of BOS, which is the hallmark of CLAD, is higher among patients who developed PGD grade 3 after lung transplantation[141].

14.3 Acute allograft rejection (AR)

Despite improvements in the field of immunosuppressive regimens, the incidence of AR after lung transplantation is highest in the first year, occurring in up to 30% of patients, and the mortality rate within 30 days is about 4%. AR is the major risk factor for the subsequent development of CLAD[130, 142].

The ISHLT has established diagnostic and grading criteria for acute allograft rejection based on the degree of lymphocytic infiltration in transbronchial biopsy from grade A0 to grade A4[143]. CT imaging appearance in AR is of poor sensitivity and specificity, such as multifocal ground-glass opacities, consolidations,

accompanied by pleural effusions but CT plays an important role in localisation of the transbronchial biopsy target area.

AR can be cell mediated or antibody mediated. Overall, cell-mediated rejection is much more common. It is mediated by T-lymphocytes in the recipient that recognise leukocyte antigens (HLAs) or other antigens in the donor graft. Cell-mediated rejection can be seen on transbronchial biopsy, characterised by lympho-histiocytic inflammatory infiltrate central to small blood vessels[130, 142]. A multicentre prospective study of 400 lung transplant patients confirmed that the degree of HLA mismatch was associated with the occurrence of AR and was significantly reduced in single lung transplantation compared to bilateral lung transplantation[144].

14.4 Chronic lung allograft dysfunction (CLAD)

14.4.1 Definition and Grading

CLAD is defined as a substantial and persistent decline (≥ 20%) in measured forced expiratory volume 1 (FEV_1) value from the reference (baseline) value for ≥ 3 weeks' post-transplantation. The baseline value is calculated as the mean of the best two postoperative FEV_1 measurements[75, 145]. The initial ''chronic'' in the term's CLAD means ''persists for a long period of time'' or ''irreversibility''[75, 145, 146].

14.4.2 Classification or phenotypes

14.4.2.1 Restrictive allograft syndrome (RAS)

The incidence of RAS among CLAD patients is 25-35%. RAS is characterised by diffuse fibrotic processes across different anatomical compartments including the airways, pleura, peripheral lung tissue and vasculature causing peripheral consolidation, as seen on radiological examination[76] *(Figure 9A).*

14.4.2.2 Bronchiolitis obliterans syndrome (BOS)

The incidence of BOS among CLAD patients is 75-85%. BOS is used as a synonym of chronic rejection presenting as obstructive bronchiolitis with hyperinflation mosaic attenuation on radiological finding. BOS is classified into four grades according to the ISHLT classification[76, 146-148] *(Figure 9B) (Table 5).*

BOS should be suspected when a previously stable patient develops dyspnoea, cough, fever and/or fatigue. Evaluation should include spirometry and radiographic imaging. In BOS, spirometry shows obstructive physiology, defined as a FEV_1 less than or equal to 80% of the mean of the two best post transplantation values taken at least 3 weeks apart.

High-resolution computed tomography (HRCT) may identify pleuro-parenchymal changes and/or air trapping. Bronchoscopy with bronchoalveolar lavage and transbronchial lung biopsy is helpful for excluding infection or other entities, such as acute cellular rejection, but it has poor predictive value for BOS[146].

Table 5. BOS grades according to ISHLT classification.

BOS 0	FEV_1 > 90% of baseline and $FEF_{25–75}$ > 75% of baseline
BOS 0-P	FEV_1 81% to 90% of baseline and/or $FEF_{25–75}$ ≤ 75% of baseline
BOS 1	FEV_1 66% to 80% of baseline
BOS 2	FEV_1 51% to 65% of baseline
BOS 3	FEV_1 50% or less of baseline

FEV_1 forced expiratory volume during the first second, $FEF_{25–75}$, mid-expiratory flow rate

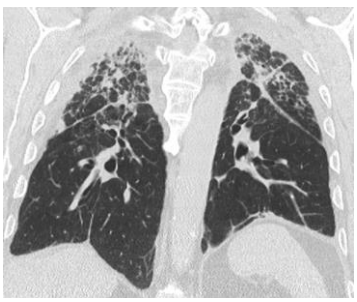


Fig. 9A. Restrictive allograft syndrome (RAS).

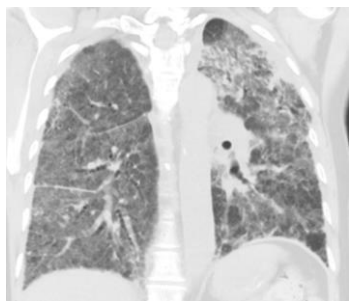


Fig. 9B. Bronchiolitis obliterans syndrome (BOS).

TABLE 6 Typical signs of complications after transplantation based on chronological order

Complications	Onset	CT signs	Clinical features
Hyperacute rejection	<24 h	- Diffuse opacities of the graft	- Acute dyspnea
PGD	<1 week	- Basal airspace consolidations - Interstitial opacities - Peribronchial and intralobular septal thickening - Little pleural effusion	- Dyspnea - The ratio of P/F combined with the imaging presentation is used for PGD grading (0–3)
Acute rejection	1 week to 1 year	- Multifocal ground-glass lesions - Lobular septal thickening - Consolidations - Pleural effusion	- Dyspnea - Cough - Lower extremity edema
BOS	>6 months	- Air trapping and mosaic attenuation - Bronchiectasis and bronchial wall thickening - Tree-in-bud and lobular central nodules	- Obstruction - FEV1 ≤ 80% baseline - FEV1/FVC ratio <0.70
Mixed	>6 months	- Concurrent obstructive and restrictive pulmonary imaging signs	- Combined obstructive and restrictive spirometric changes
RAS	>1 year	- Ground-glass opacities - Apical and upper lung fibrosis - Pleural thickening - Traction bronchiectasis - Hilar retraction and structural distortion - Volume loss	- Restriction - FEV1 ≤ 80% baseline - TLC<90% baseline

Abbreviations: BOS, bronchiolitis obliterans syndrome; CT, computed tomography; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; P/F, partial pressure of arterial oxygen (PaO2) to fraction of inspired oxygen (FiO2); PGD, primary graft dysfunction; RAS, restrictive allograft syndrome; TLC, total lung capacity.

15 *Ex vivo* lung perfusion (EVLP)

15.1 Brief history of EVLP development

The year of 1935 was the time of the first successful *ex vivo* perfusion reported when *Alexis Carrel* and *Charles Lindbergh* perfused a cat thyroid gland and ovaries using *ex vivo* perfusion for approximately 20 days[149].

The technique of EVLP was proposed initially in 1987 by *Hardesty* and *Griffith*[150]; thereafter in the 1990s, Professor *Stig Steen* and colleagues developed an EVLP platform to evaluate lung function and published the first article of using EVLP to evaluate a lung from a non-heart-beating donor before lung transplantation in 2000[12].

In 2005, *Professor Stig Steen* and colleagues performed the first human double lung transplant in the world using nonacceptable donor lung after reconditioning using an *ex vivo* technique[151].

In 2009, *Ingemansson et al.* published the results from the first six double lung transplantations performed with donor lungs that were rejected for transplantation by the Scandiatransplant, Eurotransplant, and UK transplant organisations in our clinic[152].

In 2009, the Toronto Lung Transplant Group introduced the Toronto EVLP protocol[153].

In 2010, *Lindstedt et al.* published world first comparative outcome review of double lung transplantation using conventional donor lungs and non-acceptable donor lungs reconditioned *ex vivo*[154].

In 2011, the Toronto Lung Transplant Group published the results of 20 successfully transplanted cases with donor lungs which were re-evaluated using EVLP[155].

In 2012, the first-in-human experience using the portable Organ Care System (OCS) lung device for concomitant preservation, assessment, and transport of donor lungs was reported[156].

EVLP is not just platform for reconditioning, but can also be used to administer therapeutic interventions such as antibiotics, fibrinolytics, and immune-modulators[157]. The recent multicentre NOVEL trial which compared controlled donation after circulatory death (cDCD) and donation after brain death (DBD) lung transplants using EVLP as well as a control group without EVLP showed similar rates of PGD at 24 hours, 48 hours, and 72 hours after transplant. Additionally, long-term survival between EVLP-recovered DBD and DCD allografts was similar to that of non-EVLP controls[158]. EVLP is recommended for uncontrolled donation after circulatory death (uDCD) according to the International Conference on Organ Donation's new recommendation[159].

15.2 *Ex vivo* perfusion system

EVLP is a significant advancement in donor lung preservation. EVLP is a concept that consists of supporting the donor lungs outside the human body through ventilation and perfusion with cellular or acellular solutions while sustaining sterility, humidity, and graft temperature in a closed environment.

The EVLP system includes a ventilator, an endotracheal tube, a membrane oxygenator with a built-in heat exchanger, a centrifugal pump, reservoir, and a leukocyte–arterial filter *(Figure 10).*

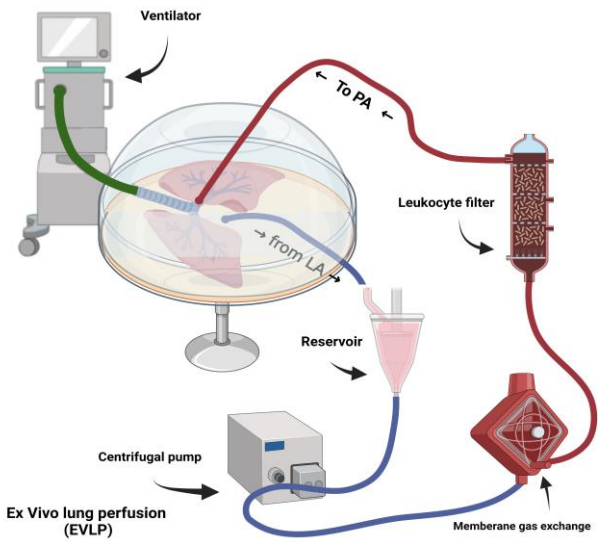


Fig. 10. *Ex vivo* lung perfusion (EVLP).
Created with BioRender.com

The system is primed with 2.0 L of Steen solution (*Vitrolife AB, Gothenburg, Sweden*) mixed with ABO-compatible, packed RBC to a haematocrit of 15%, to which is added Imipenem 0.5 g (*Tienam; Merck Sharp & Dohme, Sollentuna, Sweden*), insulin 20 IU (*Actrapid; Novo Nordisk, Bagsvaerd, Denmark*), and heparin 10,000 IU (*Leo Pharma, Malmö, Sweden*).

The Steen solution is a buffered extracellular solution that includes human albumin to provide an optimal colloid osmotic pressure, so that physiological pressure and flow can be maintained without development of pulmonary oedema.

The ventilation gas in the lung membrane consists of nitrogen (N_2) (86%), CO_2 (8%), and O_2 (6%)[160].

The two most clinically relevant available devices in clinical trials are the XVIVO Perfusion System (XPS) (*XPS Perfusion, Göteborg, Sweden*) and the OCS (*Transmedics, Andover, MA*)[161] *(Figure 11).*

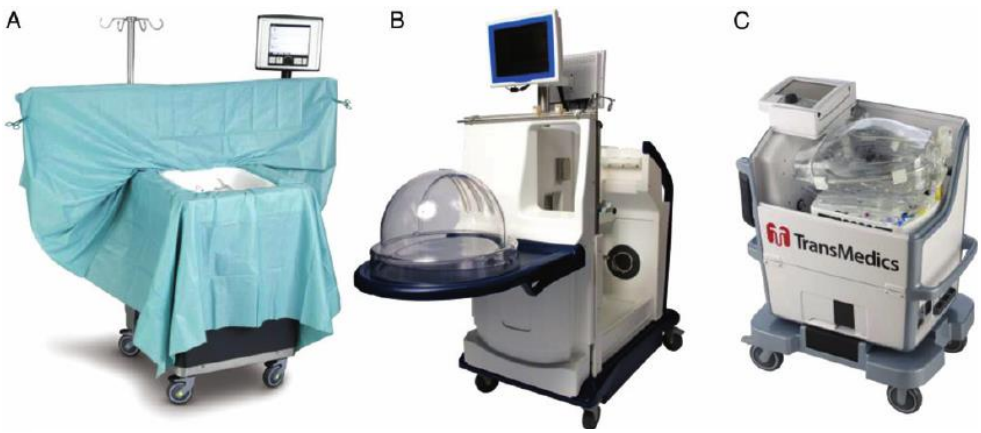


Fig. 11. The devices in clinical trials.
A Vivoline® LS1, (Vivoline Medical AB), static EVLP with the Lund EVLP protocol.
B XPS TM, (XVIVO Perfusion AB), static EVLP with the Toronto EVLP protocol.
C Organ Care System (OCS).
©XVIVO Perfusion AB

15.3 EVLP protocols

Currently, there are three EVLP systems and protocols: the clinically applied Toronto protocol, Lund protocol, and the OCS.

The Toronto system is the world's most widely used system. The Lund system is an extension of the original EVLP protocol. The OCS is the only portable EVLP system[162] *(Table 7).*

Table 7. Three EVLP systems and protocols applied clinically.

Parameter	Toronto	Lund	OCS
Perfusion			
Target flow	40% CO	100% CO	2.0–2.5 L/min
PAP	Flow dictated	≤20 mmHg	≤20 mmHg
LA pressure (mmHg)	3-5 (cloused LA)	0 (open LA)	0 (open LA)
Perfusate	Steen™ solution	Steen™solution + RBCs hct 15%	OCS™solution + RBCs hct 15–25%
Ventilation			
Start temp (°c)	32	32	34
Tidal volume	7 mL/kg bw	5–7 mL/kg bw	6 mL/kg bw
RR (BPM)	7	8	10
PEEP	5 cm H_2O	5 cm H_2O	5–7 cm H_2O
FIO_2 (%)	21	50	12

CO, cardiac output; FiO_2, inspired fraction of oxygen; hct, haematocrit; LA, left atrium; PAP, pulmonary artery pressure; RBCs, red blood cells; bw, body weight donor; bpm, breaths per minute; RR, respiratory rate; PEEP, positive end-expiratory pressure; Temp, temperature

15.1 Indications for EVLP

EVLP is currently used mainly to assess and recondition certain marginal donor lungs if they unacceptable according to the standard ISHLT donor criteria[67, 163]. The common inclusion criteria for EVLP therapy are PaO_2/FiO_2 lower than 300 mmHg, bronchoscopy findings concerning aspiration or pneumonia or pulmonary oedema, significant infiltrates on chest X-ray, massive pulmonary embolism, and lungs from donors who have suffered cardiac death[164].

The common exclusion criteria to prevent useless EVLP therapy are mechanical lung damage (tears) leading to air/blood leaks, aspiration (gross, gastric), massive lung contusion, pneumonia, purulent secretions, sepsis, multiple RBC transfusion, suspected tumours, COPD, asthma, emphysema, pleural disease, recipient <18 years, ABO incompatibility, and previous open thoracic surgery[165, 166,167].

15.2 Acceptance opinions after EVLP

There is no international guideline for acceptance criteria after reconditioning to decide whether the lungs are suitable for transplantation or not, but there are some recommendations and opinions regarding EVLP after 4-6 hours, such as:

Gas exchange at the end of the evaluation phase:
There is currently no universally accepted threshold but the common accepted measurements are[68]:
PaO_2/FiO_2 >350 mmHg with PaO_2 measured in a blood sample from the left atrium. This cut-off value varies between teams, ranging from 300 to 400 mmHg.
PCO_2 <6 kPa (45.6 mmHg) and PO_2 >50 kPa (380 mmHg) at FiO_2 =1.0.

Macroscopic and haemodynamic evaluation
For most of the transplantation centres, haemodynamic, ventilatory parameters have to remain stable and standard such as stable or improving pulmonary artery pressure (PAP), airway pressure, and pulmonary compliance. In addition, there should be absence of mass/nodules on palpation and no abnormality on bronchoscopy[68].

15.3Summary of reviewed literature on EVLP

EVLP has thus been applied successfully into clinical practice and research worldwide, with a resulting expansion of the donor lung pool (*Table 8*).

Steen *et al.* developed an *ex vivo* lung method in the mid-1990s and this new technique led to the first human lung transplantation from a non-heart-beating donor in 2000 after successful evaluation *ex vivo*. Five years later, in May 2005, the same team in our clinic performed the first human transplant of initially nonacceptable lung after *ex vivo* lung "reconditioning" as a first case worldwide. The details of this technique were published in 2007[151]. In 2009, *Ingemansson et al*. published the initial outcome of first six patients in the world who received double transplanted reconditioned lungs. The 3-month survival was 100%. One mortality occurred after

95 days due to sepsis and another mortality after 9 months due to rejection. Four recipients survived without any sign of BOS 2 years after the transplantation[152].

In November 2010, *Lindstedt S. et al*. published a comparative outcome review of the first six patients in the world who received reconditioned lungs using EVLP and the patients who received conventional lungs during the same short period in our clinic. The study showed no significant difference regarding mechanical ventilation support, time in the ICU or total hospital stay[154].

In 2011, *Cypel M. et al*. reported the results from the first prospective nonrandomised clinical trial, the "HELP" trial (*Human Ex vivo Lung Perfusion*) and in this trial a total of 136 lungs were transplanted. Lungs from 23 high-risk donors were reconditioned following 4 hours' EVLP and, among these, 20 lungs (87%) were accepted for transplantation. The other 116 lungs were considered as the control group. The study did not report any significant differences regarding the incidence of PGD as well as 30-day mortality. No significant differences were observed in ICU or hospital stay[155].

In 2012, *Aigner et al.* published the results of a prospective study of nine initially rejected donor lungs that were reconditioned by EVLP assessment, and compared these to 119 standard-preservation transplants. The study showed no 30-day mortality between the groups and no significant differences regarding ICU and hospital stay[165].

Similar outcomes were achieved by *Zych et al*. in a retrospective study which compared six EVLP recipients with 86 standard-preservation transplants[168].

An excellent report of 125 transplantations after EVLP assessment was presented by the Toronto, Paris and Vienna groups at the ISHLT meeting in 2013. The report showed that 85% of lungs reconditioned by EVLP were transplanted successfully with excellent outcomes[169].

In 2014, *Sanchez et al.* published, the NOVEL lung trial, a multicentre (six centres), prospective, non-randomised clinical trial comparing reconditioned EVLP lungs versus standard-criteria lungs. The study demonstrated that early and mid-term post-transplantation outcomes were equivalent in both groups[170, 171].

In 2016, *Fisher et al*. reported the outcomes of DEVELOP-UK, a nonrandomised observational study that compared transplantation outcomes between reconditioned extended-criteria lungs versus standard-criteria lungs, in addition to assessing the clinical- and cost-effectiveness of the EVLP treatment method. Among 53 evaluated and reconditioned donor lungs, only 18 (34%) lungs were transplanted. A total of 184 participants received standard-donor lungs. The main conclusion of this study was that the patients who received EVLP-reconditioned lungs had a higher rate of

early graft injury as well as risk for unplanned ECMO support, which is an expensive treatment[172].

In 2016, *Yeung et al.* published a Toronto Lung Transplant Programme database retrospective study comparing the outcomes between two groups. The first group involved 97 patients who received lungs preserved for more than 12 hours (including EVLP time), while the second group comprised 809 patients transplanted with lung preserved for less than 12 hours. The primary post-transplant outcomes were similar between the groups regarding risk of development of PGD at 72 hours and no significant difference in ICU/hospital stay. These results are well supportive of the concept that lung transplants can now be performed across larger geographical zones without any increase in the risk of deterioration in the outcome[173].

In 2019, *Gabriel L et al*. presented the results of the EXPAND trial, the first multicentre prospective international trial (involving centres in USA, Germany and Belgium) to evaluate a normothermic portable EVLP system (OCS). Ninety-three donor lungs from extended-criteria donors and donors after circulatory death were assessed by the OCS. Of these, 12 lungs did not meet the OCS transplantation criteria, and two lungs were excluded as a result of logistical reasons so a total of 79 patients underwent lung transplantation. The EXPAND trial reported that the 30-day mean lung-graft-related serious adverse events per patient were similar to those in patients receiving standard-criteria donor lungs regarding acute rejection, bronchial anastomotic, and major pulmonary infection[174].

Very recently, *Mallea M. et al*. published a non-randomised, multicentre (seven centres in the USA) study which evaluated the safety of extending graft preservation using a centralised lung evaluation system (CLES) based on the Toronto *ex vivo* system. A total of 115 recipients were included in the study: 63 allografts were accepted for transplantation, 66 received allografts after EVLP-CLES facility and 49 underwent standard transplant as the control group. The study showed that recipients of allografts assessed by a CLES had a higher rate of PGD3 during the first 72 hours' post-transplantation, but had similar 30-day and 1-year outcomes compared to conventional lung recipients[175].

Table 8. Summary of reviewed literature on *ex vivo* lung perfusion (EVLP)**.**

Year	Author	Title	Publication Title
2001	Steen, S.	Transplantation of lungs from a non-heart-beating donor	Lancet (London, England)[12]
2006	Wierup, Per	*Ex Vivo* evaluation of nonacceptable donor lungs	The Annals of Thoracic Surgery[176]
2009	Cypel, M.	Normothermic *ex vivo* perfusion prevents lung injury compared to extended cold preservation for transplantation	American Journal of Transplantation[153]
2009	Ingemansson, Richard	Clinical transplantation of initially rejected donor lungs after reconditioning *ex vivo*	The Annals of Thoracic Surgery[152]
2010	Sandra Lindstedt	Comparative outcome of double lung transplantation using conventional donor lungs and non-acceptable donor lungs reconditioned *ex vivo*	CardioVascular and Thoracic Surgery[154]
2011	Cypel, M.	Normothermic *ex vivo* lung perfusion in clinical lung transplantation	New England Journal of Medicine[155]
2011	Moradiellos, F. J.	90 Clinical lung transplantation after *ex vivo* evaluation of uncontrolled non heart-beating donors lungs: initial xperience	The Journal of Heart and Lung Transplantation[177]
2011	Lindstedt, Sandra	Comparative outcome of double lung transplantation using conventional donor lungs and non-acceptable donor lungs reconditioned *ex vivo*	Interactive Cardiovascular and Thoracic Surgery[154]
2012	Cypel, M.	Experience with the first 50 *ex vivo* lung perfusions in clinical transplantation	Journal of Thoracic and Cardiovascular Surgery[178]
2012	Aigner, C.	Clinical *ex vivo* lung perfusion—pushing the limits	American Journal of Transplantation[165]
2012	Wigfield, C. H.	Successful emergent lung transplantation after remote *ex vivo* perfusion optimization and transportation of donor lungs	American Journal of Transplantation[179]
2012	Valenza, F.	Extracorporeal lung perfusion and ventilation to improve donor lung function and increase the number of organs available for transplantation	Transplantation Proceedings[180]
2012	Dark, J. H.	323 Successful transplantation of unusable donor lungs using *ex-vivo* lung perfusion: the Newcastle experience	The Journal of Heart and Lung Transplantation[181]
2012	Warnecke, Gregor	Normothermic perfusion of donor lungs for preservation and assessment with the Organ Care System Lung before bilateral transplantation: a pilot study of 12 patients	The Lancet[156]
2012	Zych, Bartlomiej	Early outcomes of bilateral sequential single lung transplantation after *ex-vivo* lung evaluation and reconditioning	The Journal of Heart and Lung Transplantation[168]

Table 8. *Cont.*

2013	Cypel, M.	Three center experience with clinical normothermic *ex vivo* lung perfusion	The Journal of Heart and Lung Transplantation [169]
2013	Hopkins, P. M.	Australia's experience with *ex-vivo* lung perfusion of highly marginal donors	The Journal of Heart and Lung Transplantation[182]
2014	Valenza, Franco	*Ex vivo* lung perfusion to improve donor lung function and increase the number of organs available for transplantation	Transplant International[69]
2014	Sage, Edouard	Lung transplantation from initially rejected donors after *ex vivo* lung reconditioning: the French experience†	European Journal of Cardio-Thoracic Surgery[57]
2014	Wallinder, Andreas	Early results in transplantation of initially rejected donor lungs after *ex vivo* lung perfusion: a case-control study	European Journal of Cardio-Thoracic Surgery[183]
2014	Boffini, Massimo	Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with ex vivo lung perfusion	European Journal of Cardio-Thoracic Surgery[184]
2014	Sanchez, P. G.	The NOVEL lung trial one-year outcomes	The Journal of Heart and Lung Transplantation[171]
2015	Fildes, James E.	Clinical outcome of patients transplanted with marginal donor lungs via *ex vivo* lung perfusion compared to standard lung transplantation	Transplantation[185]
2015	Tikkanen, J.M.	Functional outcomes and quality of life after normothermic *ex vivo* lung perfusion lung transplantation	The Journal of Heart and Lung Transplantation[186]
2015	Bozso, Sabin	Lung transplantation from donors after circulatory death using portable *ex vivo* lung perfusion	Canadian Respiratory Journal[187]
2015	Mohite, P. N.	Utilization of the organ care system as *ex-vivo* lung perfusion after cold storage transportation	Perfusion[188]
2015	Machuca, T. N.	Lung transplantation with donation after circulatory determination of death donors and the impact of *ex vivo* lung perfusion	American Journal of Transplantation[189]
2016	Wallinder, A.	Transplantation after *ex vivo* lung perfusion: A midterm follow-up	The Journal of Heart and Lung Transplantation[190]
2016	Fisher, Andrew	An observational study of Donor *Ex Vivo* Lung Perfusion in UK lung transplantation: DEVELOP-UK	Health Technology Assessment[172]
2016	Zeriouh, Mohamed	Utilization of the organ care system for bilateral lung transplantation: preliminary results of a comparative study	Interactive Cardiovascular and Thoracic Surgery[191]
2016	Jonathan C Yeung	Outcomes after transplantation of lungs preserved for more than 12 h: a retrospective study	Lancet Respiratory Medicine 2017; 5: 119–24[173]
2017	Slama, Alexis	Standard donor lung procurement with normothermic *ex vivo* lung perfusion: A prospective randomized clinical trial	The Journal of Heart and Lung Transplantation[192]
2017	Luc, J. G. Y.	Feasibility of lung transplantation from donation after circulatory death donors following portable *ex vivo* lung perfusion: A pilot study	Transplantation Proceedings[193]

Table 8. *Cont.*

2018	Warnecke, Gregor	Normothermic *ex-vivo* preservation with the portable Organ Care System Lung device for bilateral lung transplantation (INSPIRE): a randomised, open-label, non-inferiority, phase 3 study	The Lancet. Respiratory Medicine[194]
2018	Koch, Achim	One-year experience with *ex vivo* lung perfusion: Preliminary results from a single center	The International Journal of Artificial Organs[195]
2019	Divithotawela, Chandima	Long-term outcomes of lung transplant with *ex vivo* lung perfusion	JAMA Surgery[196]
2019	Loor, Gabriel	Portable normothermic *ex-vivo* lung perfusion, ventilation, and functional assessment with the Organ Care System on donor lung use for transplantation from extended-criteria donors (EXPAND): a single-arm, pivotal trial	The Lancet. Respiratory Medicine[174]
2019	Nilsson, Tobias	Lung transplantation after *ex vivo* lung perfusion in two Scandinavian centres	European Journal of Cardio-Thoracic Surgery[197]
2019	Ghaidan, Haider	Ten-year follow-up of lung transplantations using initially rejected donor lungs after reconditioning using *ex vivo* lung perfusion	Journal of Cardiothoracic Surgery[198]
2019	Zhang, Zhang L.	First experience with *ex vivo* lung perfusion for initially discarded donor lungs in the Netherlands: a single-centre study	European Journal of Cardio-Thoracic Surgery[199]
2020	Chan, Patrick G.	*Ex vivo* lung perfusion: A review of research and clinical practices	Seminars in Cardiothoracic and Vascular Anesthesia[70]
2020	Fumagalli, Jacopo	Early pulmonary function and mid-term outcome in lung transplantation after *ex-vivo* lung perfusion - a single-center, retrospective, observational, cohort study	Transplant International[200]
2020	Leiva-Juárez, Miguel M.	Extended post-*ex vivo* lung perfusion cold preservation predicts primary graft dysfunction and mortality: Results from a multicentric study	The Journal of Heart and Lung Transplantation[201]
2021	Okahara, Shuji	Common criteria for *ex vivo* lung perfusion have no significant impact on posttransplant outcomes	The Annals of Thoracic Surgery[202]
2021	Undurraga M, Felipe	Lung transplant with *ex vivo* lung perfusion, Chilean and Latin American first experience	Revista Medica De Chile[203]
2022	Abdoul, Naïssa	Impact of *ex vivo* lung perfusion on brain-dead donor lung utilization: The French experience	American Journal of Transplantation[147]
2022	Nakajima, Daisuke	Successful lung transplant cases with *ex vivo* lung perfusion assessment of extended criteria donor lungs	General Thoracic and Cardiovascular Surgery[71]
2022	Mallea, Jorge M.	Remote *ex vivo* lung perfusion at a centralized evaluation facility	The Journal of Heart and Lung Transplantation[175]
2022	Peel, John Kenneth	Determining the impact of *ex-vivo* lung perfusion on hospital costs for lung transplantation: A retrospective cohort study	The Journal of Heart and Lung Transplantation[204]

16 Animal models of lung injury

The first known use of animal models in scientific experiments was during the 6th-5th centuries BC in ancient Greece. During that period, vivisections of living animals were practised widely in order to obtain knowledge about the mechanisms and functions of living organisms *(Figure 12).*

For centuries, animal experimentation has been the standard practice for learning about medicine and biology and understanding the underlying pathogenetic mechanisms[205].

Fig. 12. *"A physiological demonstration with vivisection of a dog,"* by Émile-Édouard Mouchy. This 1832 oil painting — the only secular painting known of the artist — illustrates how French scholars valued physiological experimentation in the service of scientific progress[90]. Notice how the struggling of the animal does not seem to affect the physiologist or his observers.

Currently part of the Wellcome Gallery collection, London. Source: Wellcome Library.

Different animal models of experimental lung injury have been used to investigate and understand the mechanisms of lung injury.

Mouse models of human disease are widely used in this field due to the availability of certain laboratory reagents and markers as well as the development of genetically modified mice that can be used to assess the physiological and pathological function of specific genes.

Transgenic mice have been used widely in the study of ALI and ARDS which have a relatively low cost when compared to the expensive large animal models, such as pigs or sheep, which require advanced equipment and often anaesthesiological expertise.

Ideally, animal models of ALI and ARDS should reproduce the pathophysiological mechanisms of ALI in humans. Over the years many different lung injury models have been tested.

In general, the most common direct causes of ARDS can be pneumonia, aspiration, breathing high concentrations of smoke or chemicals, and near drowning. Indirect or non-pulmonary causes are sepsis, major trauma, pancreatitis and transfusion-related ALI[115, 119].

Pneumonia and aspiration of gastric contents are the main reasons for direct lung injury, while sepsis is the major cause of indirect lung injures[115, 206].

17 Aims

Paper I

To explore the hypothesis that cytokine adsorption filtration during EVLP, and extracorporeal haemofiltration post-transplant, can restore pulmonary function and reduce the incidence of PGD.

Paper II

During 2005-2006 the first double lung transplant in the world was performed using marginal donor lungs evaluated by EVLP, which was developed by *Professor Stig Steen*. Our study presented 10 years' follow up comparing EVLP lungs with the conventional lungs performed at our clinic in the same year.

Paper III

The influence of allograft ischaemic time (IT) on short- and long-term mortality remains under debate in the field of lung transplantation. Due to a scarcity in donors, it might be possible to improve the outcome in lung transplantation by investigating associations and characteristics in IT among different recipients. This report studied the effect of IT among different patient groups in both short- and long-term mortality in lung transplantation.

Paper IV

CLAD, and especially BOS, remains the major barrier to long-term success after lung transplantation. A biomarker in blood that can diagnose BOS would be of great clinical value. In the current study we conducted broad proteomics analysis to detect biomarkers for BOS.

Paper V

To explore the hypothesis that mesenchymal stromal cells (MSCs) during EVLP and post-transplantation would restore the aspiration-damaged lung function and decrease the incidence of PGD at 72 hours' post-transplantation.

18 Materials and methods

18.1 Papers I, V

18.1.1 *Ex vivo* lung perfusion

EVLP was performed using Vivoline LS1 (XVIVO perfusion, Gothenburg, Sweden). We placed the harvested lungs *en bloc* in an EVLP dome and perfused them with 40% of cardiac output at 37ºC. Ventilation was started when the temperature reached 32°C–34°C, 7 mL/kg body weight of the donor tidal volume, 40% FiO_2, respiratory rate (RR) of seven breaths/minute. PEEP of 5 cmH_2O for 4 hours after reaching 32°C.

The system was primed with Steen™ Solution (XVIVO perfusion) and with RBCs from the donor animal, drawn prior to lipopolysaccharide (LPS) treatment or gastric juice, to reach a haematocrit level of 15–20% in the EVLP circuit. If the perfusate level in the reservoir dropped below 300 mL, additional Steen solution (XVIVO Perfusion) was added.

EVLP physiology was recorded hourly during the 4-hour perfusion period. After 4 hours of EVLP, the lungs were cooled down to 10°C for about 60 minutes before transplantation (*Figure 13*).

Fig. 13. *Ex vivo* lung perfusion (EVLP) setup. Lungs connected to *ex vivo* lung perfusion (EVLP).
Photo: Evamarie Braf

18.1.2 Cytokine adsorption (Cytosorb^{TM})

In Paper I, the ARDS donors' lungs were treated with extracorporeal cytokine adsorber which is a new technology which was approved and developed in Europe in 2011. It was designed to reduce inflammatory mediators, furthermore, it is effective in the removal of endotoxins and cytokines during sepsis and lung injuries. Many studies have shown that it has a positive impact on orthotopic heart transplantation and kidney transplantation[207].

Cytosorb® cartilage is a non-pyrogenic (endotoxin free), sterile single-use filter which contains biocompatible *polystyrene divinylbenzene copolymer* beads capable of adsorbing molecules of medium molecular weight using a combination of size exclusion and hydrophobic interactions[208] (*Figure 14*).

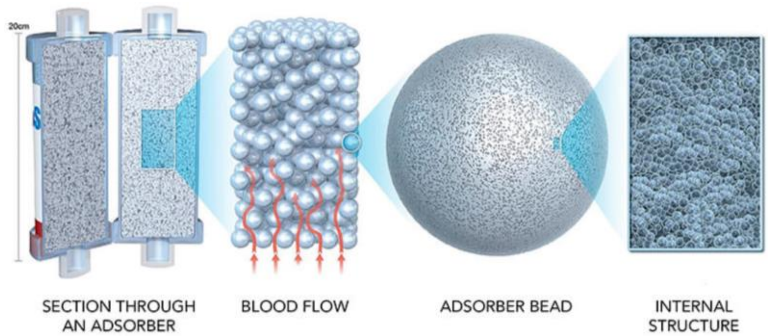


Fig. 14. Cytosorb® cartilage cross section shown adsorber bead.
© Copyright, CytoSorbents Europe GmbH

18.1.2.1 Cytokine adsorption during EVLP

In the two-steps treated animal group, the EVLP perfusate was filtered continuously through an absorbent filter (CytoSorb®, CytoSorbents Europe GmbH, Berlin, Germany) through a veno-venous shunt from the reservoir at a rate of 300 mL/min then a further 12 hours of extracorporeal haemoadsorption following transplantation (*Figure 15B*).

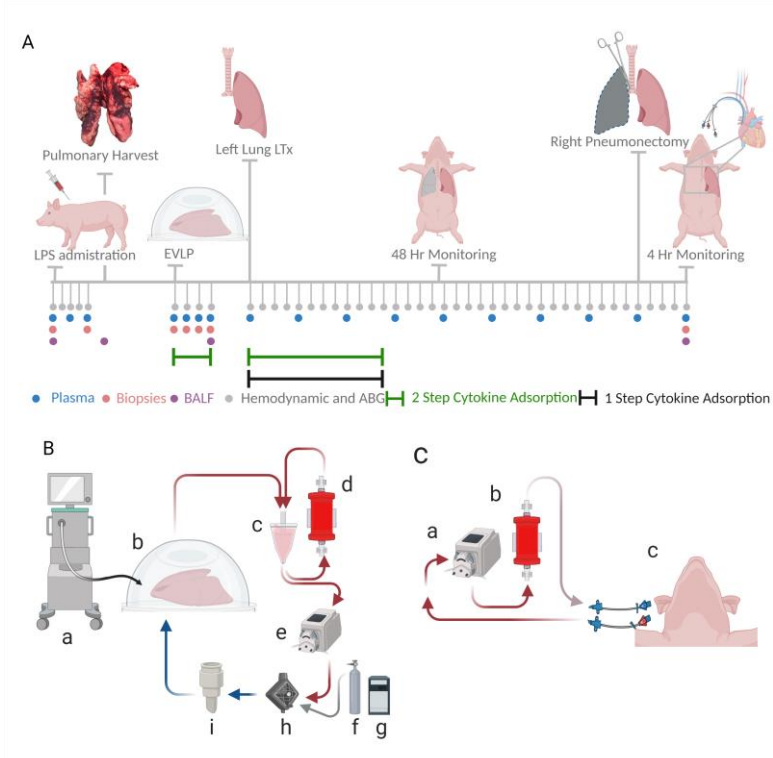


Fig. 15. Experimental overview and technique for cytokine adsorption and lung transplantation.

A Timeline of (LPS)-induced (ARDS) lung injury and lung recovery by therapeutic interventions during EVLP and transplantation (LTx) follow-up.The recipient was monitored for 48 hours after left lung transplantation and a mid-sternotomy followed by a right pneumonectomy in the last 4 hours allowed for isolated monitoring of the transplanted lung.

B. Setup of cytokine adsorption during EVLP

A mechanical ventilator (a) was connected to the lungs in the dome (b). Flow of perfusate continued into the reservoir (c) which fed into the cytokine adsorber (d) that then directed adsorbed perfusate back into the reservoir. Flow continued as per established methodology using a peristaltic pump (e) into a deoxygenator (h) connected to a gas supply (f) and heater (g). Following the leukocyte filter (i), the perfusate returned to the lungs.

C Setup of cytokine adsorption post-transplantation. A veno-venous shunt using a haemodialysis catheter was inserted into the jugular vein. This facilitated flow through a pump (a) that was in line with the cytokine adsorber (b). After adsorption, flow returned to the circulation via the haemodialysis catheter in the jugular vein.

Created with BioRender.com.

18.1.2.2 Extracorporeal haemoadsorption after transplantation

The one-step treated animal group did not receive any cytokine treatment during the EVLP, but they received cytokine absorbent filter through a veno-venous shunt for 12 hours via extracorporeal haemoadsorption following transplantation using a haemodialysis catheter (Power-Trialysis® Slim-Cath™, Becton, Dickinson and Company, New Jersey, USA) inserted in the venous jugulars with a roller pump at a rate of 300 mL/min (*Figure 15C*).

18.1.3 Treatment with mesenchymal stromal cells (MSCs)

In Paper V, the ARDS donors' lungs were treated with MSCs. Human bone marrow was harvested from 20-to-25-year-old healthy donors. The MSCs were purified and maintained in culture and propagated using a Good Manufacturing Practices (GMP)-grade animal serum-free culturing protocol. The MSCs were kept frozen at -150°C until 1 hour before use. The treated group received MSCs at a dose of 2×10^6 cells per kg body weight at three time points: during EVLP, then 1 hour and 12 hours after transplantation. The non-treated group received placebo in the form of phosphate-buffered saline (PBS) at the same time points. Each dose was given during a 30-minute time frame to avoid increasing PVR (*Figure 16*).

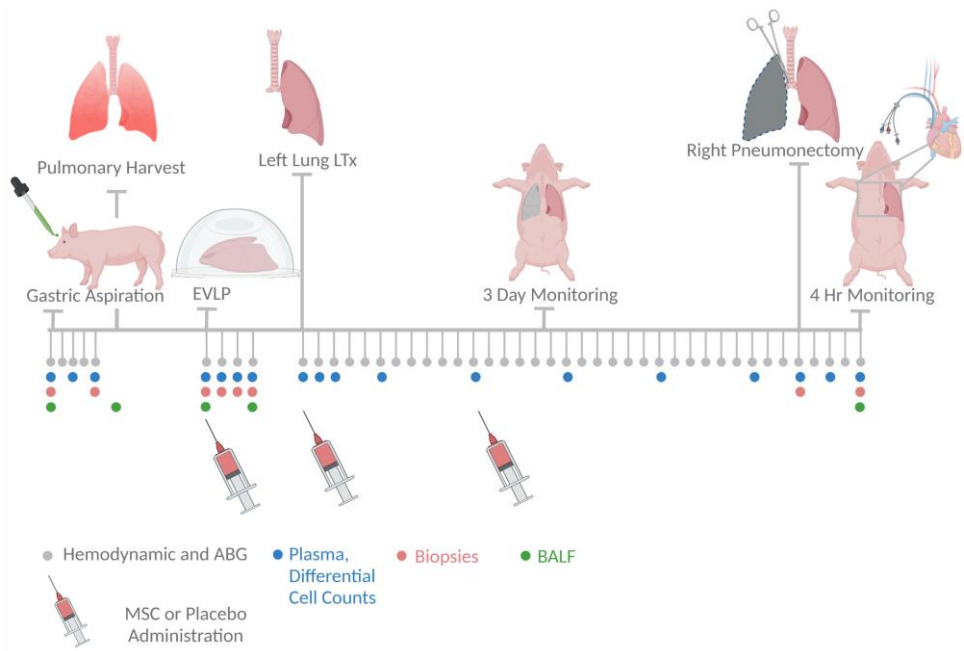


Fig. 16. Experimental overview. Timeline for lung injury induction by instillation of gastric content and administration of mesenchymal stromal cells or placebo.

Created with BioRender.com

18.1.4 Analysing cytokines in plasma

In Papers I and V during LPS or gastric aspiration and then EVLP, blood samples were collected hourly then following transplantation every fourth hour post-transplantation. Blood samples were centrifuged, and plasma separated and frozen at -80°C until analysis. From plasma samples the cytokine levels were analysed with the multiplex kit Cytokine & Chemokine 9-Plex Porcine ProcartaPlex™ Panel 1 (Thermo Fisher Scientific Cat. No. EPX090-60829-901) according to the manufacturer's instructions. The kit was run using a Bioplex-200 system (BioRad, Hercules, CA, USA). Nine cytokines were analysed: interleukin 1 beta, IL-4, IL-6, IL-8, IL-10, IL-12p40, IFN-α, interferon gamma (IFN-g), and TNFα.

18.1.1 Analysing cytokines in BALF

In Papers I and V, bronchoalveolar lavage fluid (BALF) was collected through bronchoscopy in the donor animals (during LPS and gastric aspiration) before lung harvest, at the end of EVLP, and at the end of the experiment in the donated lungs of the recipient (from left lung). The BALF was frozen at -80°C until analysis.

The multiplex kit, specifically designed for the porcine model, is an immunoassay based on the principles of a sandwich enzyme-linked immunosorbent assay (ELISA), which uses two layers of specific antibodies binding to different epitopes of one antigen (i.e. target molecule). The detection of an antigen was visualised with fluorescence using a Luminex instrument, creating a spectral signature using laser, to quantitate all protein targets simultaneously.

18.1.2 Blood cell counts

Blood cell counts were taken every 30 minutes in the donor animals (during LPS and gastric juice aspiration) then hourly throughout EVLP, and then every 1-6 hours post-transplantation. Total white blood cell counts, leukocytes and neutrophils were measured using a Sysmex KX-21N automated haematology analyser (Sysmex, Milton Keynes, UK).

Blood samples anti-coagulated with ethylene diamine tetra-acetic acid were kept at 4°C until analysis.

18.1.3 Histology

18.1.3.1 Histological process

In Paper I before administration of LPS and through sternotomy, a baseline lung biopsy was taken from the right lung then after confirmed ARDS. In both Papers I and V, biopsies were taken from the right lower lobe after the lung was harvested.

When the lung was connected to EVLP, biopsies were taken from the right lower lobe in the beginning as baseline biopsies and then hourly throughout EVLP. Additional biopsies were also taken from the transplanted left lung at end of the experiment.

Biopsies were fixed in 10% neutral buffered formalin solution (Sigma Aldrich, Germany) at 4°C overnight. Formalin-fixed tissues were subjected to a graded ethanol series and iso-propanol (both Fisher Scientific) prior to paraffin embedding (Histolab Products AB, Gothenburg, Sweden). 4 μm sections were cut and, after deparaffinisation, the sections were stained with haematoxylin and eosin (Merck Millipore, Germany) followed by dehydration in consecutively graded ethanol and xylene solutions. Dried sections were mounted with Pertex (Histolab). Brightfield images were acquired with a Nikon Eclipse Ts2R microscope (Nikon, Tokyo, Japan) (*Figure 17*).

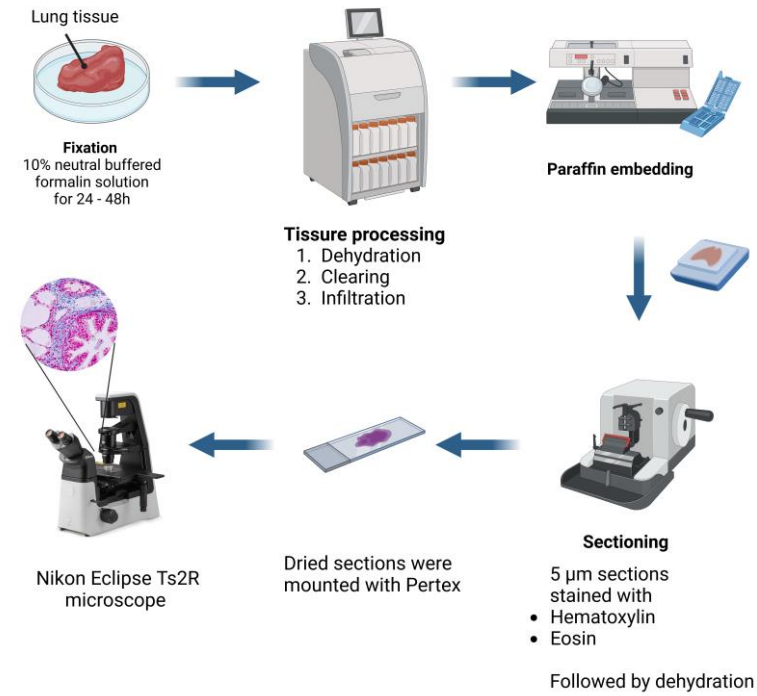


Fig. 17. The histological process of biopsies.
Created with BioRender.com

18.1.3.2 Scoring

To confirm the degree of lung injury, the histological images from each animal were scored independently for lung injury by three blinded scorers, who assessed several features such as: number of inflammatory cells; presence of hyaline membranes; level of proteinaceous debris; thickening of the alveolar wall; enhanced injury; haemorrhage; atelectasis. Scores were given a scale of 0 to 8 for each feature and reported as an average of the sum of the characteristic scores[209].

18.1.3.3 TUNEL assay

In order to assist the late **apoptosis** (Programmed cell death) in lung biopsies and give the score of injuries, TUNEL (*TdT-mediated dUTP nick end-labelling*) is one of the best methods. It is used widely to identify and quantify apoptotic cell and DNA fragmentation.

Samples were selected randomly from each group, with five slides each from the baseline and confirmed ARDS groups, as well as six slides from the EVLP groups. All slides from the end of observation of transplanted recipients were stained.

TUNEL-positive cell counts per piece were determined and normalised to the lung tissue area represented in the TUNEL score using Fiji ImageJ 1.53 M software[210].

18.1.3.4 Wet dry-weight ratio

In order to evaluate the degree of pulmonary oedema, the *wet dry-weight ratio* is used widely. Lung tissue biopsies after 4 hours' EVLP and after 48 hours' post-transplantation were weighed then freeze-dried for 24 hours, and then weighed again. The ratio between the wet and dry weight was then calculated.

18.2 Paper IV

18.2.1 Proximity extension assay (PEA)

In Paper IV, we included patients who were at least 2 years following transplantation in a stable condition with no known infection or progression of disease state. Plasma samples were collected at the time of registration in the study as a baseline samples were then followed by another sample 1 year later. All samples were collected in EDTA tubes, centrifuged, and kept frozen at -80ºC.

Proximity extension assay **(PEA**). A total of 644 proteins in plasma were analysed using **Olink Multiplex** to assess cell regulation, inflammatory, immune response, organ damage development, cardiovascular II, and cardiovascular III panels (Olink, Uppsala, Sweden, https://www.olink.com). The panels were chosen on the basis of coverage for a wide array of potential targets related to cell regulation, inflammation, immune response, and organ damage.

Each panel contains 92 antibody probe pairs that bind target proteins in the sample.

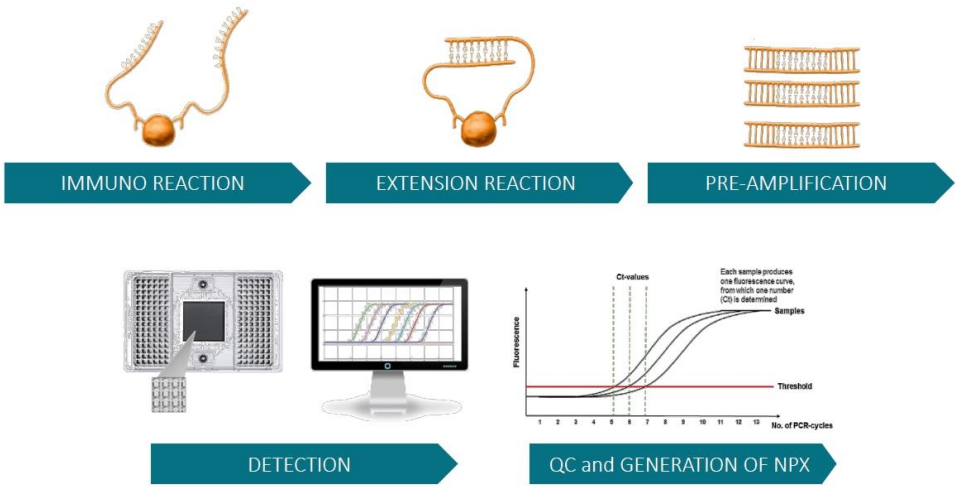
In order to validate the PEA results, CRH and MMP-9 in plasma were measured by ELISA kits according to the manufacturer's instructions:

CRH ELISA kit (*OKEH00623*) Aviva Systems Biology, San Diego, CA, USA.

HUMAN MMP9 ELISA Kit (*ab246539*), Abcam, Cambridge, UK.

The kits rely on standard sandwich ELISA technology using specific antibodies. Sensitivity of the CRH and MMP9 assays were 4.9 pg/mL and 10 pg/mL, respectively *(Figures 18-20).*

Plasma samples were taken at baseline following double lung transplantation and, of those 46 patients, 32 were analysed again after 1 year. Six patients were excluded due to re-transplantation secondary to BOS, another five died, and three were lost to follow up.



Olink Proteomics

Fig. 18. Proximity Extension Assay (PEA) technology. Each biomarker is addressed by a matched pair of antibodies, coupled to unique, partially complementary oligonucleotides, and measured by quantitative real-time PCR.

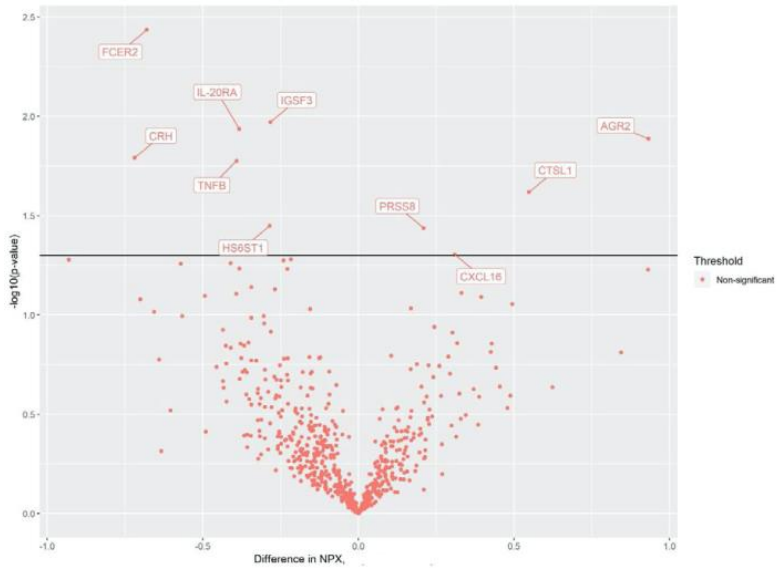


Fig. 19. Shows a volcano plot of the 644 proteins analysed using the proximity extension assay. A linear regression model compared the two groups with the solid line indicating a p value of 0.05. Proteins on the positive x-axis have higher NPX values in the BOS grade 1–3 group, and proteins on the negative x-axis have higher NPX values in the BOS grade 0 group.

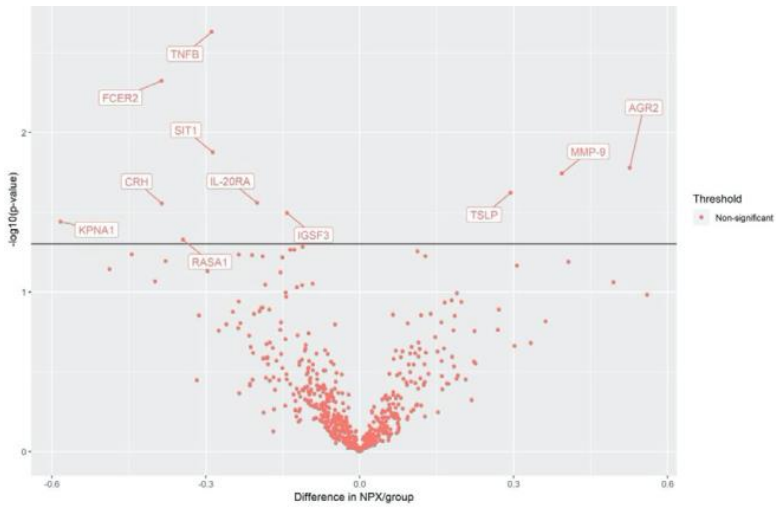


Fig. 20. Shows a volcano plot of the 644 proteins analysed using Olink proteomics. A linear regression model was conducted with the solid line depicting a p-value = 0.05. The named proteins in the plot have a p-value < 0.05. Proteins on the positive x-axis have higher NPX values in the BOS grade 2–3 group, and proteins on the negative x-axis have higher NPX values in the BOS grade 0 group.

19 Subjects and study design

19.1 Paper I

This study was a preclinical prospective, randomised study involving a total of 32 domestic pigs with a mean weight of 50 kg. Sixteen pigs used as donors were induced with ARDS via administration of LPS and 16 pigs were used as recipients. After ARDS was established via injection of LPS intravenously as an infusion (2 µg/kg/min) for 1 hour, and the dose was reduced by 50% for another hour, randomisation was instigated during 4 hours of EVLP with or without cytokine adsorption, and then followed by left lung transplantation with or without cytokine adsorption during the first 12 hours' post-transplantation as the following groups:
Non-treated groups: lungs with LPS-induced ARDS receiving EVLP and lung transplantation without cytokine adsorption (n = 6).
One-step treated group: lungs with LPS-induced ARDS receiving EVLP without cytokine adsorption but with cytokine adsorption for the first 12 hours' post-lung transplantation (n = 4).
Two-step treated: Lungs with LPS-induced ARDS receiving cytokine adsorption during EVLP and again for the first 12 hours' post-lung transplantation (n = 6).

The aim was to restore pulmonary function to make the lungs suitable for lung transplantation and reduce the postoperative risk of PGD (*Figure 15*).

19.2 Paper II

This was a retrospective study presenting 10 years of follow up of the first six double lung transplants in the world using marginal donor lungs evaluated by using EVLP developed by *Professor Stig Steen*. Here we compared EVLP lungs with the conventional lung transplants performed at our clinic in the same year. Pulmonary function was measured with spirometry and 6MWT at 3, 6, 12 months and annually. Kaplan–Meier and Cox regression analyses were used to assess survival and freedom from CLAD.

19.3 Paper III

This was a retrospective study of 307 patients who underwent lung transplantation at Lund University Hospital, Sweden between January 1990 and June 2016.

Allograft IT was defined as the mean elapsed time between cross-clamp of the aorta at organ harvest until reperfusion during transplantation. Clinical characteristics were divided into two different IT groups (IT ≤ 240 minutes and IT > 240) and subgroups IT (≤120, 121-240, 241-360 and 361+ minutes).

This report studied the effect of IT among different patient groups in both short- and long-term mortality in lung transplantation assessed by Cox regression and Kaplan–Meier survival. The endpoint used was death or re-transplantation.

19.4 Paper IV

This was a cohort study of 46 patients who underwent double lung transplantation in our clinic at Lund University Hospital, Sweden, and they were in stable condition over 2 years from transplant and without ongoing infection.

Plasma was collected and analysed for protein biomarkers using a multiplex immunoassay at baseline and at 1 year.

A total of 46 lung transplant recipients were selected who had verified CLAD with phenotype BOS based on pulmonary function tests, chest imaging, and transbronchial biopsies according to the ISHLT guidelines. Those with restrictive allograft syndrome (RAS) were excluded.

Plasma samples were collected at the time of register in the study from patients at least 2 years following transplantation who were in a stable condition with no known infection or progression of disease state. Baseline samples were then followed by another sample 1 year later. All samples were collected in EDTA tubes, centrifuged, and kept frozen at -80ºC.

19.5 Paper V

This study was a preclinical prospective, randomised study involving a total of 24 Yorkshire pigs with a mean weight of 50 kg.

Twelve pigs used as donors were induced with ARDS via endotracheally-administered gastric content and 12 pigs were used as recipients. After ARDS was established, randomisation was instigated during 4 hours of EVLP with or without MSCs, and then followed by left lung transplantation with or without MSCs as the following groups:

Treated group: ARDS lungs treated with 4 hours EVLP following by left lung transplantation receiving MSCs during EVLP and post lung transplant (n = 6).

Non-treated group: ARDS lungs treated with 4 hours EVLP following by left lung transplantation receiving placebo during EVLP and post lung transplant (n = 6).

The recipient was kept under anaesthesia for 72 hours' post transplantation. The last phase consisted of an isolated assessment of the transplanted lung following a right pneumonectomy. Treatment effect was assessed by haemodynamic and pulmonary function responses.

The aim was to restore pulmonary function to make the lungs suitable for lung transplantation and reduce the postoperative risk of PGD (*Figure 16*).

20 Statistical analysis

20.1 Paper I

Continuous variables were reported as mean ± SEM. Statistically significant differences between groups were tested with the Student's t-test and within groups with analysis of variance when data were distributed normally. The Mann–Whitney test and the Wilcoxon test were used when data were not distributed normally. A Chi-squared test was performed to analyse observed frequencies of categorical variables. All statistical analysis was performed using GraphPad Prism Software 51 version 8, (San Diego, CA, USA). Significance was defined as: $p<0.001$ (***), $p<0.01$ (**), $p<0.05$ (*), and $p>0.05$ (not significant).

20.2 Paper II

Data were presented as mean with standard deviation (SD), median with range, or frequency with percentage. The Shapiro–Wilks test was used to determine which variables were normally distributed/parametric (mean, SD) versus non-normally distributed/non-parametric (median, range). Independent (unpaired) Student's t-test was conducted for normally distributed continuous variables while the Mann–Whitney U (Wilcoxon rank sum) test was used for non-normally distributed continuous data. The Chi-squared test or Fisher's exact test were chosen for analysis of categorical variables. For survival analysis, the endpoint used was death or re-transplantation. For freedom from BOS analysis, the endpoint used was occurrence of BOS (grade ≥ 1) until death/re-transplantation/follow-up. Cox regression in accordance with Cox proportional hazards model was performed for univariable survival analysis and freedom from BOS analysis. Survival/freedom from BOS estimates were displayed in accordance with Kaplan–Meier with log-rank test to detect significance between survival/freedom from BOS curves. A p-value < 0.05 was considered to be statistically significant.

Statistical analyses were performed using SPSS Version 24.0 (IBM Corp., Armonk, NY, USA).

20.3 Paper III

Data were presented as mean (SD), median (range), or frequency (percentage). Missing data were estimated using multiple imputation[96]. The Shapiro–Wilks test was conducted to determine normally distributed (mean, SD) versus non-normally distributed (median, range) variables. Unpaired Student's t-test was used for continuous variables, except when data were non-normally distributed, in which case the Wilcoxon test was conducted instead.

For categorical variables, Chi-squared/Fisher's exact test was conducted. For survival analyses, re-transplantation-free survival was the chosen endpoint. Cox regression estimates in accordance with Cox proportional hazards model were conducted for univariable/multivariable survival analysis.

The Kaplan–Meier test was chosen to display survival estimates in addition to the Log-Rank test to detect significance between survival curves. Survival curves were truncated when fewer than 10% of the respective cohorts remained. Heart-lung transplant patients were excluded for all survival analyses.

A p-value < 0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS Version 25.0 (IBM Corp., Armonk, NY, USA).

20.4 Paper IV

Proximity extension assay (PEA) data were presented as median (minimum and maximum). ELISA data were presented as mean and SEM.

Statistically significant differences were determined by Student's t-test (normally distributed data) and by the Mann–Whitney test (non-parametric data). Analysis was performed using GraphPad Prism. Significance was defined as: $p<0.001$ (***), $p<0.01$ (**), $p<0.05$ (*), and $p>0.05$ (not significant), apart from PEA values where statistical significance was set at $p<0.01$ to counteract multiple comparisons.

20.5 Paper V

Continuous variables were reported as mean and SD. Normal distribution was tested using the Shapiro–Wilk test. Statistically significant differences between and within groups were tested with a Student's t-test. When the data were not distributed normally, nonparametric-tests were used, including the Wilcoxon test within groups and the Mann–Whitney U-test between groups. One-way ANOVA was used within groups when the data were distributed normally and the Kruskal–Wallis test when they were not distributed normally. A Chi-squared test was performed to analyse observed frequencies of categorical variables. All statistical analyses were performed using GraphPad Prism (Version 9.2, GraphPad Software, San Diego, USA). Significance was defined as *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, $p>0.05$, not significant.

21 Results

21.1 Paper I

All LPS-treated donors developed mild-to-moderate ARDS within 120 ± 30 minutes after administration of LPS intra-venously and ARDS was confirmed via two blood gases taken at a 15-minute interval. ARDS confirmation was according to the Berlin definition[120].

There was no significant difference (p=0.733) in ARDS severity between all groups.
Two-step treatment group (*PaO_2/FiO_2 ratio =208.2 ± 55.5 mmHg*)
One-step treatment group (*PaO_2/FiO_2 ratio = 204.8 ± 43.4 mmHg*)
Non-treated group (*PaO_2/FiO_2 ratio =225.3 ± 33.6 mmHg*)

All donors showed haemodynamic instability after LPS administration and required inotropic support, as shown (*Table 9*).

Table 9 Clinically relevant measurements of vitals and mechanical ventilator settings during establishment of LPS-induced ARDS for all pigs.

	Baseline ($n = 16$)	Confirmed ARDS ($n = 16$)	*p* value
Sat (%)	98.9 ± 1.4	96.1 ± 3.4	>0.9999
HR (bpm)	73.8 ± 18.2	131.8 ± 18.3	0.5670
SBP (mmHg)	101.5 ± 10.2	100.6 ± 23.4	>0.9999
DBP (mmHg)	70.6 ± 10.7	62.9 ± 24.2	>0.9999
MAP (mmHg)	83.2 ± 11.0	72.6 ± 22.6	>0.9999
CVP (mmHg)	6.8 ± 2.9	6.6 ± 2.5	>0.9999
Temp (°C)	38.5 ± 1.7	39.0 ± 2.0	>0.9999
SPP (mmHg)	25.3 ± 4.8	39.6 ± 9.4	>0.9999
DPP (mmHg)	13.5 ± 4.7	26.8 ± 8.4	>0.9999
MPP (mmHg)	18.9 ± 4.1	31.6 ± 6.8	>0.9999
Wedge (mmHg)	10.6 ± 3.4	10.4 ± 5.5	>0.9999
CO (L/min)	4.0 ± 0.9	5.8 ± 2.2	>0.9999
SVR (DS/cm^5)	1517.2 ± 312.1	1044.3 ± 405.4	**<0.0001**
PVR (DS/cm^5)	173.2 ± 68.1	364.0 ± 183.4	**<0.0001**
CI	2.8 ± 0.5	4.3 ± 1.6	>0.9999
pH	7.4 ± 0.1	7.3 ± 0.1	>0.9999
$PaCO_2$ (mmHg)	40.7 ± 6.1	54.0 ± 6.2	>0.9999
PaO_2 (mmHg)	247.3 ± 33.6	107.8 ± 24.5	**<0.0001**
Hb (g/L)	91.2 ± 10.4	95.1 ± 13.1	>0.9999
Lactate (mmol/L)	1.6 ± 0.5	2.4 ± 1.0	>0.9999
BE (mmol/L)	4.5 ± 2.7	2.1 ± 1.5	>0.9999
MV (L/min)	7.9 ± 1.1	8.5 ± 1.6	>0.9999
Max. Pressure (cmH_2O)	16.7 ± 2.6	20.4 ± 3.7	>0.9999
PEEP (cmH_2O)	5.0 ± 0.0	5.0 ± 0.0	>0.9999
Vt (mL)	363.9 ± 63.0	363.4 ± 52.9	>0.9999
C_{dyn} (mL/cmH_2O)	33.1 ± 11.6	23.8 ± 4.9	>0.9999
RR (breaths/min)	21.4 ± 3.4	23.6 ± 3.4	>0.9999
PaO_2/FiO_2 (mmHg)	494.2 ± 53.4	213.6 ± 43.0	**<0.0001**

Sat oxygen saturation, *HR* heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *CVP* central venous pressure, *Temp* temperature. Hemodynamic variables: *SPP* systolic pulmonary pressure, *DPP* diastolic pulmonary pressure, *MPP* mean pulmonary pressure, *Wedge* pulmonary artery wedge pressure, CO cardiac output, SVR systemic vascular resistance. Blood gas parameters: pH, PaO_2 partial pressure of oxygen, $PaCO_2$ partial pressure of carbon dioxide, *Hb* hemoglobin, lactate, *BE* base excess, PaO_2/FiO_2 partial pressure of oxygen divided by fraction of inspired oxygen. Mechanical ventilator settings with volume-controlled ventilation: *MV* minute volume, *PIP* peak inspiratory pressure, *PEEP* peak inspiratory pressure, positive end-expiratory pressure, *Vt* tidal volume, *Cdyn* dynamic compliance, *RR* respiratory rate, FiO_2 fraction of inspired oxygen.
Two-sided Mann–Whitney test was used for statistical analysis. *P* values less than 0.05 are highlighted in bold text.

During the induction of ARDS, plasma and BALF samples showed a significant increase of cytokines including IL-1β, IL-6, IL-8, IL-10, IL-12, and TNFα, compared to baseline and these cytokines play a critical role in ARDS and in confirming the disease model[211-213] (*Figure 21*).

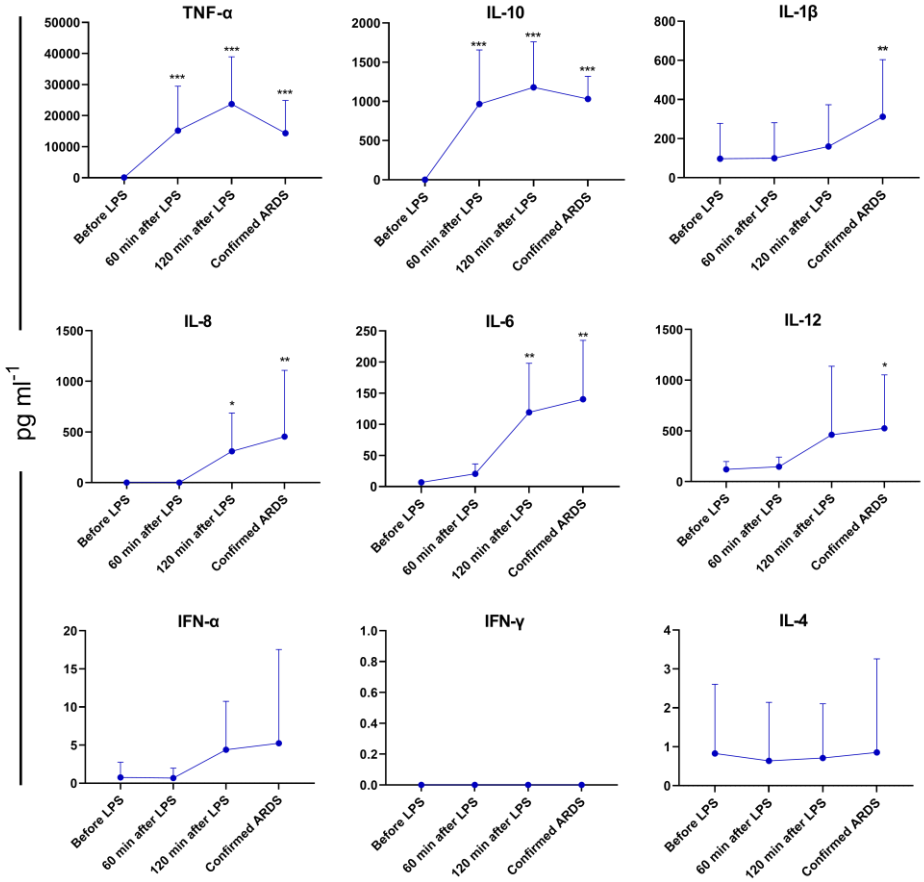


Fig. 21. Cytokine measurement in plasma in the donor before LPS was administered and then 60 and 120 min after LPS was given (n = 12). Cytokines were also measured in the plasma at the time of confirmed ARDS.

A dramatic decrease in intravascular white blood cells following LPS administration was observed (*Figure 22*).

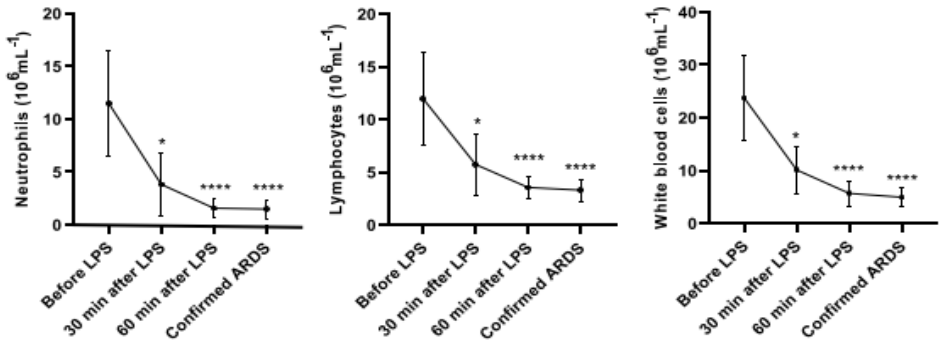


Fig. 22. Neutrophils, lymphocytes, and white blood cell counts were recorded at baseline, 30 and 60 minutes after LPS and at confirmed ARDS.

Before administration of LPS, a baseline BALF was collected via bronchoscopy and then collected again at the confirmed ARDS time point. This showed a significant increase of cytokines including IL-1β, IL-6, IL-8, IL-10 and TNFα (*Figure 23*).

A baseline lung tissue biopsy taken before LPS administration for histological analysis appeared normal, without anomalies, but following the administration of LPS, lung tissue showed significant infiltration of the alveolar spaces by immune cells, erythrocytes, also appearance of early hyaline membrane formation and atelectasis which affected most of the alveolar spaces.

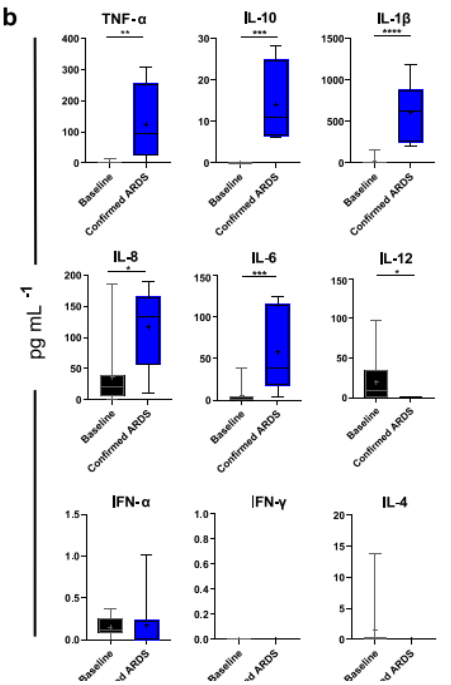


Fig. 23. Cytokine measurement in BALF which shows a significant increase of cytokines including IL-1β, IL-6, IL-8, IL-10 and TNFα during LPS adminstration and ARDS confirmation.

Furthermore, there was also vasodilation, haemorrhage, and aggregation of neutrophils (*Figure 24).*

For confirmation of lung injury analysis, blinded scoring was performed on all pigs at baseline, post-ARDS confirmation, after 4 hours' EVLP and post-transplantation by three independent observers, which showed a significant increase in cumulative lung injury score from baseline and following ARDS. No significant differences were seen between the treated group (two-step and one-step treatment) and the non-treated group at baseline.

TUNEL scoring of positive cells in baseline biopsies and biopsies taken after ARDS confirmation showed significant differences (*Figure 25*).

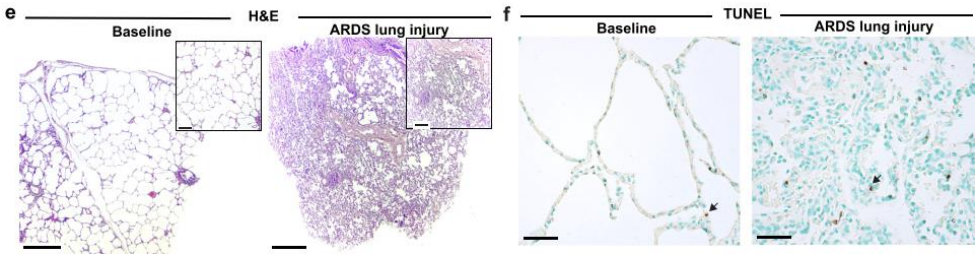


Fig. 24.

e Baseline (left) and ARDS lung injury (right) haematoxylin and eosin (H&E) staining. Scale bar in the larger image represents 0.5 mm. The callout shows a magnified portion of the tissue where the scale bar represents 0.2 mm.

f Representative images of TUNEL staining in baseline (top left) and injured lungs (bottom left) with representative black arrows indicating the type of positively stained cell counted.

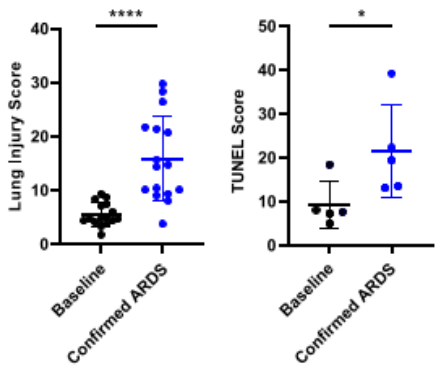


Fig. 25. Scoring of lung injury of baseline biopsies and biopsies taken at pulmonary harvest after ARDS confirmation (left) and scoring of terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) positive cells/mm^2 (right).

After harvesting, the lungs were placed in cold storage in Perfadex® PLUS solution for 2 hours before being connected to EVLP for 4 hours.

In the cytokine adsorbent-treated lungs group had an improved in gas exchange capacity and reached a PaO_2/FiO_2 ratio of 324 ± 70, which is the threshold of clinical acceptance for transplantation, while the non-treated lungs did not pass clinical acceptance as they had a PaO_2/FiO_2 ratio of 249 ± 143.

Progress in PVR occurred during the EVLP for all lung groups; however, this progress was not significant between the groups. There were no significant differences between the groups regarding the airway pressure or the pulmonary compliance (*Figure 26a*).

A significant decrease occurred in the proinflammatory cytokine IL-1β in treated lungs relative to the non-treated lungs in the perfusate and BALF. Generally lower level of cytokines could be detected in the treated lungs; however, none reached significance level (*Figures 26b-c*).

There was no significant change in the number of neutrophils, leukocytes, and total white blood count during the EVLP period in all donor groups (*Figure 26e*).

The histology of the tissue showed a significant difference between the cytokine adsorption-treated lungs and those without adsorption with regard to morphology of lung injury. Wet/dry ratio showed no significant difference between the groups at the end of EVLP.

TUNEL staining was performed and showed no difference between the treated and non-treated groups (*Figures 26f,g,h*).

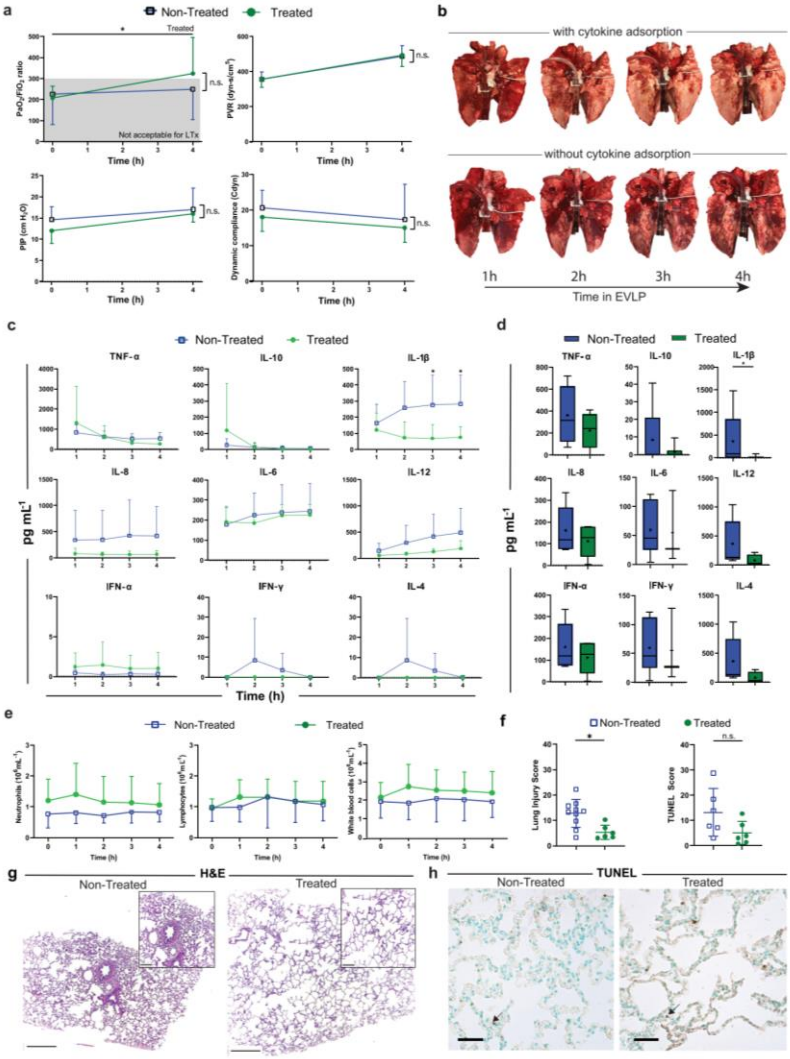


Fig. 26. Improvement of pulmonary function and inflammation following cytokine adsorption during *ex vivo* lung perfusion (EVLP) treatment. **a** Measures of the PaO_2/FiO_2 ratio, the pulmonary vascular resistance (PVR), peak inspiratory pressure (PIP), and dynamic compliance were recorded throughout EVLP. **b** Gross morphology of the treated lungs (top) and the non-treated lungs (bottom) throughout the 4-hour period. **c** Cytokines in plasma with samples taken every hour of EVLP, with 1 hour marking the time elapsed since the start of treatment (n = 6 per group). **d** The bronchoalveolar lavage fluid (BALF) was tested at the end of EVLP for cytokine levels (n = 6 per group). **e** Cell counts of neutrophils, lymphocytes, and white blood cells were measured every hour. **f** The scores of the histologycompare cytokine adsorption groups (left) and the cell counts per mm^2 after terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining (right). **g** Images representative of n = 16 samples of haematoxylin and eosin (H&E) histology of non-treated (left) lungs and treated (right). **h** Representative images of n = 5 lungs of TUNEL staining in non-treated (left) and treated lungs (right).

Statistically significant differences between non-treated and treated groups were tested with two-sided Student's t-test and within groups with ANOVA when data were distributed normally. The two-sided Mann–Whitney test and the Kruskal–Wallis test were used when data were not distributed normally. *p<0.05, **p<0.01, ***p<0.001, n.s. non significant. All values represent the mean ± standard deviation unless otherwise stated.

After 4 hours of EVLP, the left lung was subsequently transplanted. Following this, extracorporeal haemoperfusion with a cytokine adsorber filter was connected to the treated recipients (one-step treatment and two-step treatment) during the first 12 hours' post-transplantation. Both treated groups showed improved haemodynamic stability compared to the non-treated group (*Table 10*).

Cytokines were generally decreased post-transplantation in the treated group; however, none of these levels reached statistical significance (*Figures 27a,b*).

Significant decreases in both neutrophil counts and total white blood cell counts were noted in the two-step treated group, especially after the right pneumonectomy (*Figure 27c*) while they were unchanged in the one-step treated group.

The lung tissue wet/dry weight ratios were measured after 4 hours of EVLP then after 48 hours' post-transplantation in the two-step treatment and non-treatment groups and showed a significant decrease in average wet/dry ratio among the two-step cytokine adsorption-treated lung group.

Regarding the histology, in the non-treated group, there were significant morphological changes characteristic of ARDS including the accumulation of immune cells, intra-alveolar haemorrhage, and the collapse of most alveolar spaces after transplantation. In the one-step and two-step treated recipients, an immune response was still seen, represented by the infiltration of immune cells but the alveolar spaces were mostly open, and respiratory bronchioles and blood vessels appeared without major visible damage. Generally, there was a decreased score in both treated lungs relative to the non-treated ones at the 48 hours' post-transplantation biopsies (*Figures 27d,e*).

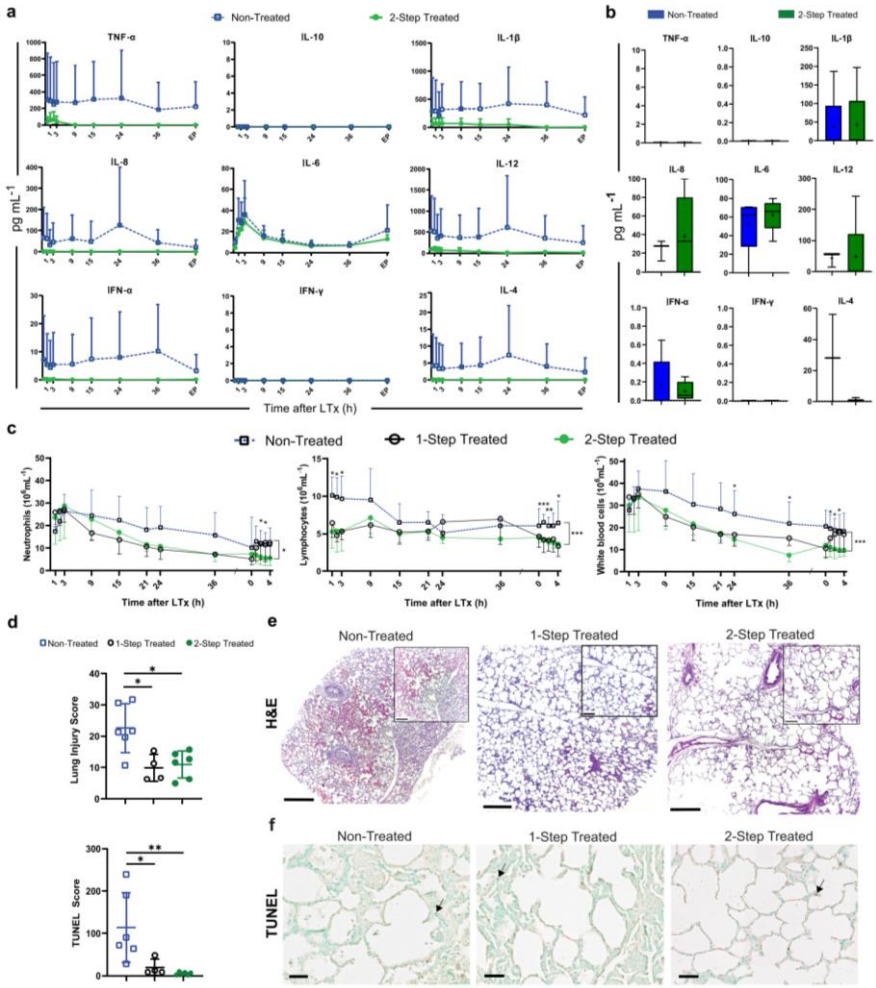


Fig. 27. Reduced inflammatory state during lung transplantation (LTx) and follow-up. **a** Plasma cytokine levels were monitored throughout the 48-hour period following transplantation, **b** Bronchoalveolar lavage fluid (BALF) was tested for cytokine concentrations at the termination of the experiment. **c** Cell counts including neutrophils, lymphocytes, and white blood cells were analysed. **d** Scoring of the lung injury across groups (top) and scoring of the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) cell counts per mm^2 (bottom). **e** Haematoxylin and eosin (H&E) staining representative of n = 16 of non-treated (left), one-step treated (middle) and two-step treated (right) biopsies taken at the end, following 4 hours of isolated transplanted lung function. **f** Representative images of n = 5 lungs of TUNEL staining in non-treated (left), one-step treated (middle) and two-step treated lungs (right) with representative black arrows indicating positively stained cells used in the TUNEL score.

Statistically significant differences between groups were tested with two-sided Student's t-test and within groups with ANOVA when data were distributed normally. The two-sided Mann–Whitney test and the Kruskal–Wallis test were used when data were not distributed normally. *p<0.05, **p<0.01, ***p<0.001. All values represent the mean ± standard deviation unless otherwise stated.

Table 10 Clinically relevant measurements of vitals and mechanical ventilator settings post transplantation for all recipients.

Time	Sat (%)	HR (bpm)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	CVP (mmHg)	Temp (°C)	pH	BE (mmol/L)	MV (L/min)	Max. Pressure (cmH_2O)	PEEP (cmH_2O)	Vt (mL)	Cdyn (mL/cmH_2O)	RR (breaths/min)
Baseline	98.2 ± 1.4	67.3 ± 14.8	120.3 ± 17.8	87 ± 14.3	100 ± 14.7	8.3 ± 4.4	37.1 ± 1	7.5 ± 0.08	6.2 ± 3.2	8.35 ± 1.5	20.8 ± 3.3	5.8 ± 2.0	396.2 ± 33.5	26.4 ± 7.7	21.5 ± 3.8
	100 ± 0	**93.7 ± 15**	**94.5 ± 9.3**	**58.2 ± 9.5**	**72 ± 10.8**	**8 ± 3.2**	**39.7 ± 1.3**	**7.4 ± 0.04**	**4.2 ± 3.8**	**7.3 ± 0.2**	**19.7 ± 2.8**	**5.5 ± 0.5**	**361.9 ± 51.2**	**30.1 ± 8.2**	**25.2 ± 0.5**
	98.3 ± 2.7	74.0 ± 16	105.3 ± 10.4	69.5 ± 7.8	83.5 ± 8.6	5.3 ± 3.5	36.8 ± 1.5	7.5 ± 0.2	5.6 ± 2.1	8.5 ± 2.0	17.7 ± 1.5	5.0 ± 0.0	388.3 ± 44.3	31.8 ± 5.4	20.7 ± 3.3
1 h	97.2 ± 2.4	88.7 ± 3.8	101.2 ± 12.4	62 ± 13	76.7 ± 14.5	5.8 ± 2.4	36.6 ± 1.9	7.3 ± 0.06	4.3 ± 3.5	10 ± 1.3	23.6 ± 2.6	6 ± 2.0	396.7 ± 53.9	24.3 ± 1.4	25.8 ± 3.9
	96.5 ± 3.8	**90.8 ± 6.8**	**102 ± 8.5**	**62.7 ± 7.5**	**79.25 ± 6.3**	**6.2 ± 3.5**	**40.7 ± 0.8**	**7.3 ± 0.05**	**3.3 ± 3**	**7.7 ± 0.6**	**27.2 ± 4**	**7.7 ± 1.5**	**413.0 ± 59**	**24.2 ± 2.7**	**28 ± 2.8**
	97.7 ± 1.7	90.8 ± 6.2	102.8 ± 4.1	67.5 ± 5	87.3 ± 5.5	4 ± 3.2	36.4 ± 2.4	7.3 ± 0.04	2.4 ± 1.3	9.3 ± 1.7	22.5 ± 2	5.8 ± 1.1	387.8 ± 38	24.3 ± 4.0	22.5 ± 2.5
12 h	97.7 ± 1.9	77.3 ± 15.3	106.3 ± 8.6	70 ± 8.7	84.5 ± 9.2	8 ± 3.9	38.5 ± 1.2	7.4 ± 0.04	5.5 ± 3.2	9.8 ± 1.5	22.5 ± 2	6.5 ± 2	404.8 ± 56.3	30.2 ± 3.4	27 ± 3
	100 ± 0	**95.5 ± 11.6**	**109 ± 12.1**	**66 ± 6.2**	**84.2 ± 8.7**	**5.7 ± 2.6**	**38.5 ± 0.9**	**7.4 ± 0.04**	**5.6 ± 1**	**8.05 ± 0.5**	**25.5 ± 2.6**	**7.2 ± 0.5**	**443.2 ± 40.3**	**20.9 ± 4.2**	**26.7 ± 1.5**
	97.8 ± 3.3	76.4 ± 17.3	103.2 ± 4.9	75 ± 19.1	82.4 ± 7.9	5 ± 2.8	38.7 ± 1	7.4 ± 0.05	3.7 ± 2.2	9.7 ± 1.7	20.2 ± 1.7	6.4 ± 1.5	408.6 ± 31.4	30.1 ± 2.8	23.23.6
24 h	98.2 ± 1	80.4 ± 13.2	107.8 ± 5.2	63.8 ± 5	79.6 ± 5.9	7.4 ± 2	38.5 ± 1.1	7.5 ± 0.1	6.6 ± 2.6	11 ± 0.8	22 ± 1.4	6.2 ± 2.1	418.3 ± 58	30.7 ± 12.9	24.5 ± 2.3
	99.7 ± 0.5	**108.7 ± 5.1**	**113.5 ± 16**	**70.5 ± 20**	**88.7 ± 18.3**	**8.7 ± 4.6**	**40.4 ± 0.4**	**7.4 ± 0.05**	**5.1 ± 1.6**	**8.05 ± 0.4**	**25 ± 2.8**	**7.2 ± 0.5**	**427.0 ± 46.3**	**27.5 ± 2.8**	**26.7 ± 1.5**
	97.2 ± 2	73.6 ± 15	100.8 ± 7.8	72.4 ± 16.8	79.2 ± 9.8	5.4 ± 2.9	39.3 ± 0.5	7.4 ± 0.08	7.5 ± 1.9	9.3 ± 0.8	20 ± 1.8	6.4 ± 1.5	413.6 ± 52.6	30.6 ± 3.5	22.6 ± 3.1
36 h	97.8 ± 2	74.2 ± 14.3	108 ± 6.2	67 ± 4.9	84 ± 5.5	7 ± 2.3	38.7 ± 1.1	7.4 ± 0.04	6 ± 2.4	10 ± 1	22 ± 1.7	6 ± 3.5	417 ± 48.4	22.8 ± 3.3	26.3 ± 2.8
	99.6 ± 0.5	**112.3 ± 2.8**	**114.3 ± 12.8**	**74.6 ± 20.1**	**91.6 ± 17.7**	**9.3 ± 4**	**40.7 ± 0.3**	**7.4 ± 0.07**	**6.5 ± 0.9**	**7.7 ± 0.8**	**26.3 ± 3.7**	**7.6 ± 0.5**	**432.9 ± 38.9**	**27.2 ± 4.2**	**26.7 ± 1.5**
	97.6 ± 3.6	75.8 ± 14.7	108.6 ± 9.4	75 ± 18.6	85 ± 11	6 ± 3.5	39.4 ± 0.4	7.4 ± 0.16	8.8 ± 1.2	10 ± 1.6	20 ± 2.0	6.4 ± 1.9	417 ± 48.1	30.2 ± 3.1	22.6 ± 2.6
48 h	97.6 ± 1	74.2 ± 13.2	102.4 ± 12.7	66 ± 12.2	82.8 ± 7.9	6.4 ± 2.7	38.8 ± 1.1	7.5 ± 0.2	6.0 ± 0.4	10 ± 1	23 ± 1.7	6.5 ± 3.5	426.7 ± 10.6	22.7 ± 1.2	25.8 ± 2.8
	99 ± 1.7	**110 ± 9.6**	**104 ± 6**	**63.3 ± 11.2**	**78.6 ± 16.4**	**8.6 ± 3.5**	**40.5 ± 0.4**	**7.4 ± 0.07**	**6.5 ± 0.9**	**7.5 ± 1.2**	**27.5 ± 4.4**	**8 ± 0.8**	**427.0 ± 46.3**	**25.9 ± 6.9**	**26.7 ± 1.5**
	97.2 ± 3.6	75.8 ± 14.7	103.2 ± 6.7	71 ± 19.7	80.8 ± 15.6	5.8 ± 2	39.3 ± 0.5	7.4 ± 0.14	6.5 ± 2.8	9.5 ± 1	21 ± 2.2	6.4 ± 1.5	412 ± 51	30 ± 3.5	22.6 ± 2.6

The mean ± SD values for two-step treated animals ($n = 6$) are shown in the first row and one-step treated animals ($n = 4$) in the second row with bold text while the non-treated animals ($n = 6$ until 12 h, $n = 5$ after 12 h) are shown in the third row for each respective timepoint. Two-step treated: First rows; One-step treated: Second rows, bold text; Non-treated: Third rows

Sat Measurements for oxygen saturation, *HR* heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *CVP* central venous pressure, *Temp* temperature, pH, *BE* base excess, Mechanical ventilator settings with volume-controlled ventilation: *MV* minute volume, *PIP* peak inspiratory pressure, *PEEP* peak inspiratory pressure, positive end-expiratory pressure, *Vt* tidal volume, *RR* respiratory rate.

All animals, whether treated or non-treated recipients, were monitored for 48 hours' post-transplantation; additionally for 4 hours with complete isolated transplanted left lung function following a right pneumonectomy *(Figure 15).*

The overview of clinically relevant vital measurements during these 4 hours is shown in (*Table 11*) and (*Figure 28*), which demonstrate improved oxygenation capacity of the lung and improved pulmonary vascular resistance of the transplanted lung alone especially after the right pneumonectomy. Additionally, a significant increase in gas exchange was noted in the two-step treated group compared with the non-treated group ($p<0.0001$) (*Figure 28a*).

Pulmonary compliance was generally improved in the two-step treated group compared to the non-treated group ($p=0.001$). Lactate was lower in the two-step treated group ($p=0.001$), (*Table 11*).

At the end of the experiment (including all recipients), the PaO_2/FiO_2 ratios became higher in both the one-step and two-step groups (*Figure 27c*). In addition, PVR was found to be significantly lower in both the one-step and two-step treated groups (*Figure 28b*).

The overview of development of PGD: in the non-treated group, five of six recipients developed PGD3; in the one-step treated group, two had PGD grade 0 and two had PGD grade 2; in the two-step treated group, only one recipient developed PGD grade 2 ($p=0.006$, *Figure 28d*).

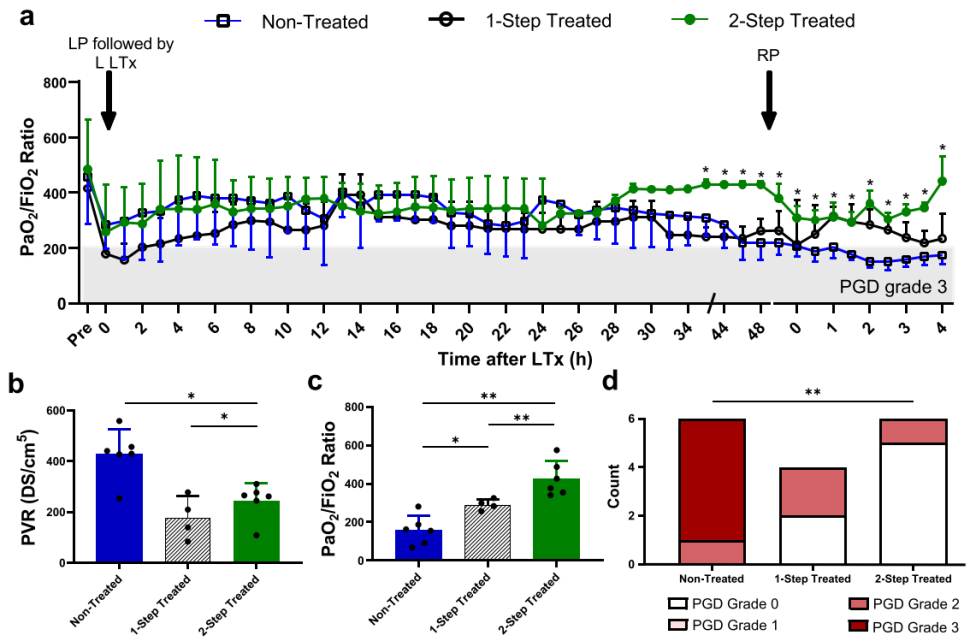


Fig. 28. Reduced primary graft dysfunction (PGD) in treated recipients**. a** PaO_2/FiO_2 ratios for all groups were followed from before transplantation in the recipient to 48 hours of follow-up. The first arrow indicates a left pneumonectomy followed by left lung transplantation (LP followed by L LTx) and the second arrow depicts the time of right pneumonectomy (RP). Statistical significance applies to direct comparison of two-step treatment to the non-treated group **b** Pulmonary vascular resistance (PVR) data (left) and **c** PaO_2/FiO_2 ratios (right) for all groups at the end of the experiment including all recipients. **d** Comparison of PGD grades following transplantation. All graphs represent data from either the two-step treated recipient lungs (n = 6), the one-step treated recipient (n = 4) or non-treated lungs (n = 6, n = 5 following 9 hours' post-transplantation). Statistically significant differences between groups were tested with two-sided Student's t-test and within groups with ANOVA when data were distributed normally. The two-sided Mann–Whitney test and the Kruskal–Wallis test were used when data were not distributed normally. Chi-squared analysis was performed to analyse observed frequencies of categorical PGD grades. *p<0.05, **p<0.01, ***p<0.001. All values represent the mean ± standard deviation.

Table 11 Overview of clinically relevant measurements of vitals and mechanical ventilator settings during the last phase of the experiment.

	Before Pneumonectomy	4 h Post Pneumonectomy	Non- vs 1-Step Treated	Non- vs 2-Step Treated	1-Step vs 2-Step Treated
Sat (%)	96.1 ± 3.3	96 ± 2.5	0.9936	0.9998	0.9952
	100 ± 0	**100 ± 0**			
	96.8 ± 1.5	95.2 ± 4.3			
HR (bpm)	91 ± 17.2	83.3 ± 19.0	0.5930	0.9857	0.4750
	107 ± 10.5	**133.3 ± 12.5**			
	79 ± 17.0	89.8 ± 34.2			
SBP (mmHg)	106.6 ± 13.1	101.6 ± 7.5	0.9931	0.9961	0.9992
	102 ± 8.2	**100 ± 9.8**			
	109.4± 8.5	105 ± 12.5			
DBP (mmHg)	63 ± 19.0	47.4 ± 17.5	0.9705	0.8935	0.9819
	61.2 ± 9.5	**55.2 ± 8.4**			
	72 ± 8.6	65.6 ± 3.1			
MAP (mmHg)	80.2 ± 20.1	64.8 ± 21.1	0.9783	0.9206	0.9866
	73.7 ± 12.6	**71.5 ± 11.6**			
	87.8 ± 11.4	80.4 ± 6.1			
CVP (mmHg)	6.8 ± 2.3	7 ± 2.5	0.9951	0.9997	0.9969
	8.7 ± 2.8	**10.2 ± 2.8**			
	7.6 ± 3.4	6 ± 3.1			
Temp (°C)	38.6 ± 0.6	38 ± 0.8	>0.9999	>0.9999	>0.9999
	39.4 ± 0.4	**38.2 ± 0.6**			
	39.5 ± 0.4	38.4 ± 1.2			
CO (L/min)	4.3 ± 0.9	3.7 ± 0.6	0.9997	0.9991	>0.9999
	4.75 ± 0.8	**4.2 ± 0.8**			
	4.5 ± 0.1	5.3 ± 0.9			
SVR (DS/cm^5)	1327 ± 356	1415 ± 413	**0.0009**	**<0.0001**	**<0.0001**
	1202.5 ± 452.2	**1192.5 ± 240.6**			
	1180 ± 200	1030 ± 139			
pH	7.4 ± 0.1	7.3 ± 0.1	>0.9999	>0.9999	>0.9999
	7.4 ± 0.05	**7.4 ± 1.0**			
	7.4 ± 0.1	7.3 ± 0.2			
Hb (g/L)	72 ± 10.2	69.4 ± 10.5	0.7691	0.9425	0.9115
	51.5 ± 4.2	**51.8 ± 2**			
	83 ± 3.4	82.6 ± 10.1			
BE (mmol/L)	6.4 ± 3.8	8.7 ± 2.7	0.9956	>0.9999	0.9945
	6.6 ± 2.8	**4.4 ± 1.2**			
	6.9 ± 4.1	8.4 ± 1.8			
MV (L/min)	9.7 ± 1.8	11.8 ± 3.0	0.9958	>0.9999	0.9952
	7.3 ± 1.2	**7.8 ± 0.8**			
	10.1 ± 1.5	11.7 ± 1.6			
Max. Pressure (cmH_2O)	22 ± 2.5	24 ± 3.0	0.9991	0.9993	0.9969
	26.7 ± 2.2	**27.2 ± 1.8**			
	22.4 ± 3.4	25.4 ± 2.9			
PEEP (cmH_2O)	6.4 ± 2.2	6.0 ± 2.2	0.9975	>0.9999	0.9980
	8 ± 0.8	**8.6 ± 1**			
	6.2 ± 1.6	5.6 ± 1.3			
Vt (mL)	407.0 ± 58.5	396.5 ± 35.4	0.7573	0.9575	0.8829
	416.5 ± 36.8	**416.9 ± 55.8**			
	429.0 ± 52.5	385.2 ± 22.5			
Cdyn (mL/cmH_2O)	26.4 ± 1.8	22.7 ± 2.4	0.9956	0.9982	0.9991
	26.0 ± 5.8	**24.4 ± 4.8**			
	27.1 ± 5.0	20.4 ± 3.4			
RR (breaths/min)	26.4 ± 4.1	22.7 ± 3.3	>0.9999	0.9830	0.9863
	26 ± 1.4	**29.5 ± 4**			
	23.8 ± 4.0	29.8 ± 4.9			
SPP (mmHg)	27 ± 6	38 ± 3	0.9989	>0.9999	0.9988
	30 ± 6	**36 ± 6.7**			
	26 ± 4	38 ± 8			
DPP (mmHg)	18 ± 11	23.5 ± 3	0.9705	0.9948	0.9874
	16 ± 2.9	**17 ± 4.8**			
	15 ± 6.0	27.4 ± 12.0			
MPP (mmHg)	24 ± 2.0	31 ± 2	0.9849	0.9997	0.9791
	20.7 ± 4.3	**22.6 ± 5.9**			
	24 ± 2.9	30 ± 8.2			
Lactate (mmol/L)	1.2 ± 0.3	1.3 ± 0.2	>0.9999	>0.9999	>0.9999
	0.9 ± 0.3	**1.5 ± 0.2**			
	1.3 ± 0.4	1.2 ± 0.4			

The values for the two-step treated recipients (n = 6) are shown in the first row, one-step treated recipients (n = 4) in the second row with bold text, and the non-treated recipients (n = 5) are in the third row for each respective parameter. Mann–Whitney and Kruskal–Wallis tests were used for statistical analysis. P values less than 0.05 are highlighted in bold text. Two-step treated: First rows (n = 6); One-step treated: Second rows, bold text (n = 4); Non-treated: Third rows (n = 5). Sat: oxygen saturation, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, CVP: central venous pressure, Temp: temperature, Haemodynamic variables: SPP: systolic pulmonary pressure, DPP diastolic pulmonary pressure, MPP mean pulmonary pressure, CO cardiac output, SVR: systemic vascular resistance, PVR: pulmonary vascular resistance, Blood gas parameters: Hb: haemoglobin, lactate, BE: base excess, Mechanical ventilator settings with volume-controlled ventilation: MV: minute volume, PIP: peak inspiratory pressure, PEEP: peak inspiratory pressure, positive end-expiratory pressure, Vt: tidal volume, RR: respiratory rate.

21.2 Paper II

The first human double lung transplant in the world using marginal donor lungs evaluated by using EVLP was performed in May 2005 at Lund University Hospital, Sweden. Between 2006-2007, 21 patients (six EVLP, 15 conventional) underwent double-lung transplant with follow-up for 10 years. Pulmonary function follow up was measured with spirometry and 6MWT at 3, 6, 12 months and annually.

The median age for these patients was 52 years with a range of 22–66 years. Nine were males and 12 females.

The major indications for a lung transplant were: COPD (n = 8); CF (n = 8); α1-antitrypsin deficiency (AAT1) (n = 1); pulmonary fibrosis (PF) (n = 2); lymphangio-leiomyomatosis (LAM) (n = 1); and pulmonary hypertension (PH) (n = 1).

Regarding the clinical characteristics of overall recipients, there were no significant differences between EVLP and conventional lung transplant concerning pulmonary function (FVC, FEV_1, 6MWT), liver/kidney-status (AST, ALT, creatinine) and pre-operative life support (ECMO or mechanical ventilation) ($p>0.05$); moreover, no significant difference was found in post-transplantation cause of death between EVLP versus conventional lung transplant ($p>0.05$) *(Tables 12-13).*

Table 12. Recipient baseline and clinical characteristics of EVLP and conventional lung transplant.

Variables	EVLP (n = 6)	Conventional (n = 15)	p-value
Weight (kg)	70.7 ± 19.3	59.1 ± 7.9	0.060
Height (cm)	170.8 ± 11.8	169.9 ± 10.1	0.862
BMI	24.0 ± 5.3	20.5 ± 3.5	0.088
Male	3 (50%)	6 (40%)	0.523
Age (years)	54.1 ± 10.4	42.6 ± 14.8	0.100
Waiting list (days)	49.0 (7 - 174)	44 (4 - 389)	0.785
Pre-op Life support			
Mechanical ventilation	0 (0.00%)	1 (6.66%)	0.714
ECMO	0 (0.00%)	1 (6.66%)	0.750
Major indication			0.407
COPD	3 (50.00%)	5 (33.33%)	
AAT1	1 (16.66%)	0 (0.00%)	
PH	0 (0.00%)	1 (6.66%)	
CF	1 (16.66%)	7 (46.66%)	
PF	1 (16.66%)	1 (6.66%)	
LAM	0 (0.00%)	1 (6.66%)	
Lab values			
FVC (litres)	2.0 ± 0.4	1.0 ± 0.6	0.540
FEV1 (litres)	0.8 ± 10.4	0.54.1 ± 10.4	0.516
6MWT (%)	39.6 ± 21.4	45.9 ± 25.1	0.600
P-ALT (µkat/L)	0.41 ± 0.15	0.32 ± 10.4	0.181
P-AST (µkat/L)	0.46 ± 0.12	0.41 ± 0.11	0.443
P-creatinine (µmol/L)	64.4 ± 11.5	54.1 ± 15.8	0. 216
Tx-type			
SLTx	0 (0%)	0 (0%)	
DLTx	6 (100%)	15 (100%)	
HLTx	0 (0%)	0 (0%)	
Re-LTx	0 (0%)	0 (0%)	

Data are mean (SD), number (%), or median (range). The numbers are based on patients with available data. COPD: chronic obstructive pulmonary disease; AAT1: Alpha 1-antitrypsin deficiency; PH: pulmonary hypertension; CF: cystic fibrosis; PF: pulmonary fibrosis; LAM: Lymphangio-leiomyomatosis; BMI: body-mass index; FVC: forced volume vital capacity; FEV_1: forced volume expiratory capacity 1 sec; 6MWT: 6-minute walking test; AST: aspartate transaminase; ALT: alanine transaminase; SLTx: single-lung transplantation; DLTx: double-lung transplantation; HLTx: heart-lung transplantation; Re-LTx: Re-lung transplantation; ECMO: extracorporeal membrane oxygenation.

Table 13. Cause of death after transplantation between EVLP and conventional lung transplant.

	EVLP (n = 6)	Conventional (n = 15)	p-value
Cause of death			0.406
Total number of deaths	3	6	
Death from organ rejection	2 (66.66%)	2 (33.33%)	
Death from infection	0 (0.00%)	2 (33.33%)	
Death from malignancy	0 (0.00%)	1 (16.66%)	
Death from miscellaneous	1 (33.33%)	1 (16.66%)	

The group "Death from Miscellaneous" includes patients with mortality caused by myocardial and cerebral ischaemia, multiple organ failure such as renal and liver failure, as well as other causes related to the patient's age and individual health status.

Cumulative survival rate estimates at 1-, 3-, 5- and 10-years are explained in terms of percentage with an upper/lower 95% confidence interval (CI) *(Figure 29).*

EVLP group showed at 1-, 5- and 7-year survival rates of **67%** (CI 48–86), **67%** (CI 48–86), and **50%** (CI 30–70), respectively.

Conventional lung transplant group showed at 1-, 3-,5- and 7-year survival rates of **93%** (CI 87–99), **73%** (CI 62–85), **53%** (CI 40–66) and **40%** (CI 27–53), respectively ($p>0.05$).

There was no significant difference between EVLP and conventional lung transplant relating to 1-year and 5-years' survival rate ($p>0.05$).

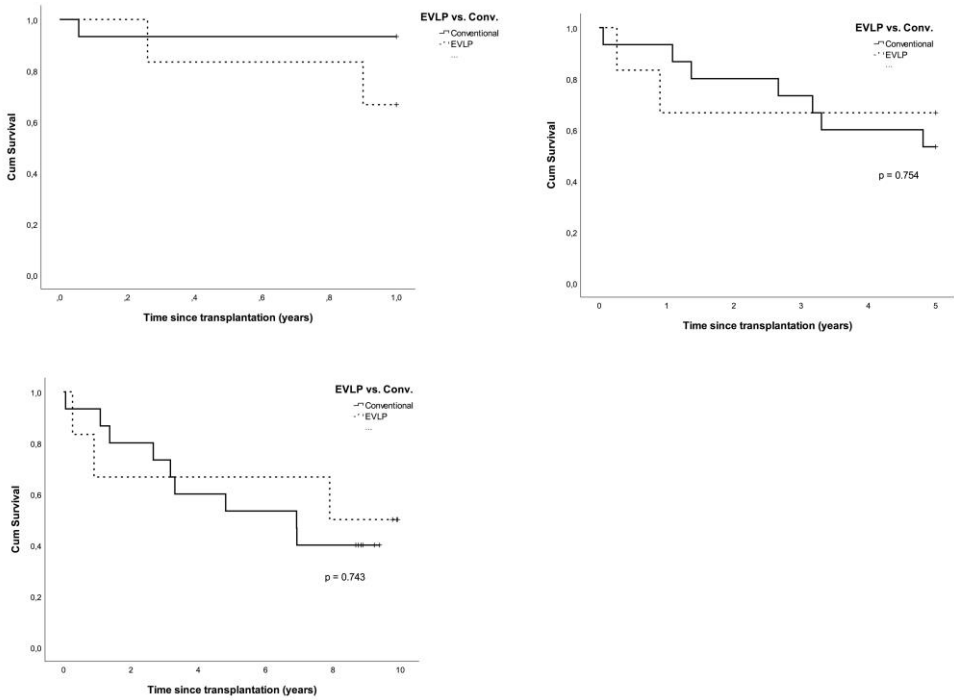


Fig. 29. The upper right Kaplan–Meier figure illustrates post-transplant survival for *ex vivo* lung perfusion (EVLP) lung transplantation (LTx) versus conventional LTx for recipients transplanted between 2006 and 2007 with a limited survival up to 1 year (p>0.05) while the upper left figure displays recipients with a limited survival up to 5 years. The bottom figure displays overall post-transplant survival in the 10-year experience for LTx-recipients (EVLP-LTx and conventional LTx) (p>0.05).

Freedom from BOS (grade ≤ 1) estimates are shown in *(Figure 30)*.

Conventional lung transplant at 1-, 3-, 5- and 7-years were **93%** (CI: 86–100), **70%** (CI: 45–94), **61%** (CI: 34–88) and **52%** (CI: 24–80), respectively.

EVLP at 1- and 3-year rates of **100%** and **75%** (CI: 53–97) respectively (p>0.05).

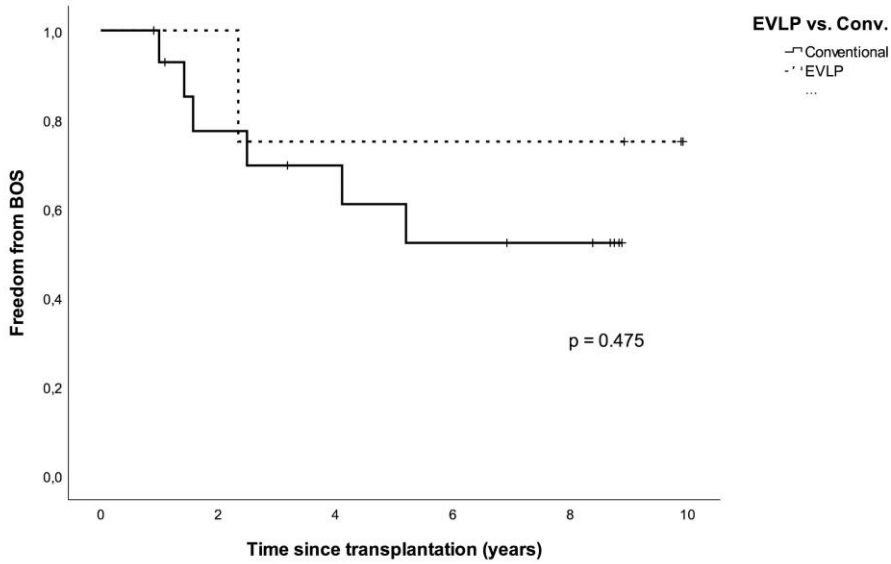


Fig. 30. Kaplan–Meier figure displaying freedom from CLAD for *ex vivo* lung perfusion (EVLP) lung transplantation (LTx) versus conventional LTx for recipients transplanted between 2006 and 2007 until follow-up or death/Re-LTx ($p<0.05$).

The Cox proportional hazards model (univariable) evaluating the survival and freedom from BOS (grade ≤ 0) for EVLP versus conventional lung transplantation is shown *(Table 14).*

No significant difference was found in overall survival up to 1-year and 5-year for EVLP vs. conventional lung transplantation ($p>0.05$).
No significant difference was found in freedom from BOS (grade ≤ 1) between EVLP and conventional lung transplantation ($p>0.05$).
Median FEV_1 and 6MWT pulmonary function with 95% CI over time is shown *(Figure 31).*

Median FEV_1 over time:
FEV_1 in EVLP group showed at 1-, 5- and 7-years, **2.1** L (1.9-2.2), **2.2** L (2.1-2.5) and **2.**1 (1.7-2.6), respectively.
FEV_1 in conventional lung transplant group showed **2.6** L (1.0-3.3), **3.0** L (0.4-4.2) and **2.9** (0.5-3.1), respectively ($p>0.05$).

Median 6MWT over time:
EVLP displayed **83%** (57-87) at 1-year, **84%** (70-112) at 5-years and **79%** (74-119) at 7-years.
Conventional lung transplantation displayed **71%** (55-79), **88%** (28-115) and **69%** (10-123) ($p>0.05$).

Table 14. Cox proportional hazards model (univariable) for EVLP and conventional lung transplant, evaluating survival and freedom from CLAD.

	HR	95 % CI	p-value
Overall survival			
EVLP	1.245	0. 335–4. 633	0.744
5-year limited survival			
EVLP	1.286	0. 266–6. 206	0.754
1-year limited survival			
EVLP	0.197	0. 018–2. 175	0.185
Freedom from CLAD			
EVLP	0.470	0. 057–3. 917	0.486

CI: confidence interval; HR: hazard ratio.

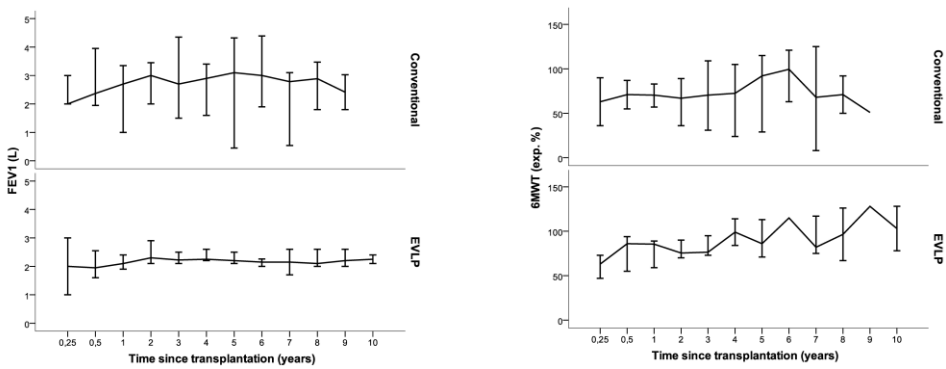


Fig. 31. Median pulmonary function with 95% confidence interval is shown over time (years) after lung transplantation (LTx). Median forced expiratory volume in 1 second (FEV1) in litres is displayed to the right while 6-min walking test (6MWT) in expected work percentage is shown to the left for *ex vivo* lung perfusion (EVLP)-lung transplant and conventional-lung transplant, respectively.

21.3 Paper III

Our retrospective study of 307 patients underwent lung transplantation at Lund University Hospital, Sweden between January 1990 and June 2016. These patients were divided into four groups according to the allograft ischaemic time (≤120, 121-240, 241-360 and 361+ minutes), (*Figure 32).*

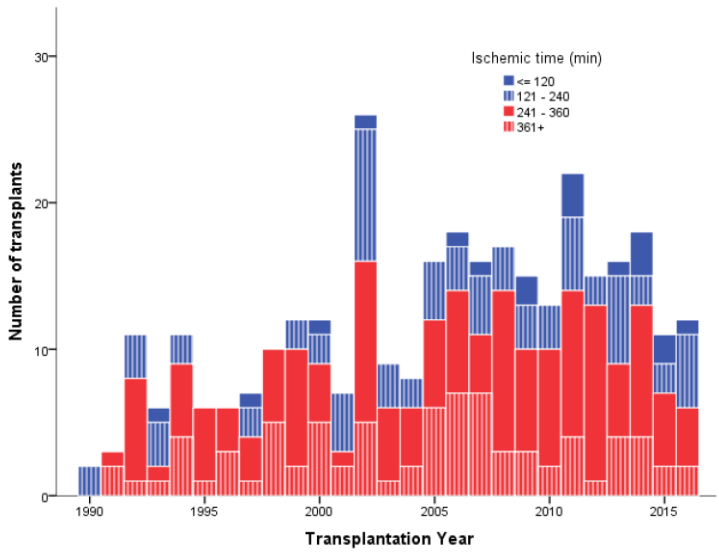


Fig. 32. Temporal distribution of all lung transplants at our single-centre stratified into minutes of allograft ischaemic time groups of ≤120 (n = 18), 121–240 (n = 79),241–360 (n = 148) and 361+ (n = 80) that occurred between January 1990 and June 2016.

The donor/recipient's clinical characteristics were divided into two different ischaemic time groups (IT ≤ 240 minutes and IT > 240 minutes).

Regarding the recipients' data, there were no significant differences between groups such as waiting list time, FEV_1, 6MWT, liver/kidney-status and pre-operative life support (ECMO or mechanical ventilation). Neither were any differences shown in major indication, BMI, nor CMV/EBV/toxoplasma-mismatch; also, no difference was found in the cause of death as well between the IT groups (*Tables 15-16).*

Table 15. Recipient/donor baseline and clinical characteristics of allograft ischemic time (IT) less/equal than 240 min and more than 241 min, respectively.

Variables	IT ≤ 240 min ($n = 96$)	IT > 240 min ($n = 229$)	p Value
Recipient data			
Recipients major indication			.195
COPD	25 (26 %)	49 (21 %)	
AAT1	23 (24 %)	36 (16 %)	
PH	14 (15 %)	30 (13 %)	
CF	15 (16 %)	43 (19 %)	
PF	10 (10 %)	34 (15 %)	
Others	3 (3 %)	25 (11 %)	
Graft failure (Re-LTx)	6 (6 %)	12 (5 %)	
ABO-identical match (yes)	72 (75 %)	190 (83 %)	.184
CMV serology (pos)	71 (74 %)	180 (79 %)	.384
EBV serology (pos)	69 (72 %)	156 (68 %)	.287
Toxoplasma serology (pos)	28 (29 %)	52 (23 %)	.201
CMV-mismatch (yes)	17 (18 %)	35 (15 %)	.511
EBV-mismatch (yes)	8 (8 %)	16 (7 %)	.571
Toxoplasma mismatch (yes)	15 (16 %)	23 (10 %)	.441
Weight (kg)	62.3 ± 11.78	63.4 ± 14.7	.557
Recipient/Donor weight ratio	0.9 (0.1–1.6)	0.9 (0.4–3.0)	.475
Height (cm)	169.6 ± 8.3	169.0 ± 9.7	.582
Recipient/Donor height ratio	1.0 (0.8–1.4)	1.0 (0.8–2.4)	.276
BMI	21.6 ± 3.7	22.1 ± 4.3	.348
Male	47 (49 %)	110 (48 %)	.715
Gender mismatch (yes)	39 (41 %)	72 (31 %)	.054
Age (years)	52 (18–72)	52 (12–71)	.273
Recipient/Donor age ratio	1.0 (0.1–3.1)	1.0 (0.1–3.9)	.887
Waiting list (days)	103 (1–1717)	77 (2.0–1305)	.114
Transplantation year > 2005 2005	52 (54 %)	121 (53 %)	.713
Lab values			
FVC (liters)	2.2 (1.0–5.3)	2.1 (0.3–5.8)	**.007**
FEV1 (liters)	0.9 (0.2–2.7)	0.9 (0.1–3.4)	.896
6MWT (%)	39.8 ± 23.3	39.0 ± 21.4	.813
P-ALT (μkat/L)	0.4 (0.1–2.3)	0.4 (0.1–9.7)	.103
P-AST (μkat/L)	0.4 (0.2–2.0)	0.4 (0.1–10.0)	.113
P-creatinine (μmol/L)	65 (32–234)	62 (27–216)	.153
Pulm. pressure > 25mmhg	30 (31 %)	74 (32 %)	.601
Tx-type			**<.001**
SLTx	43 (45 %)	57 (25 %)	
DLTx	38 (40 %)	159 (69.5 %)	
HLTx	9 (9 %)	1 (0.5 %)	
Re-LTx	6 (6 %)	12 (5 %)	
ATG (yes)	69 (72 %)	146 (64%)	.122
Pre-op Life support			
Mechanical ventilation	3 (3 %)	1 (0.5 %)	.361
ECMO	1 (1 %)	13 (6 %)	.075
Donor data			
CMV serology (pos)	50 (52 %)	161 (70 %)	**.007**
EBV serology (pos)	45 (47 %)	118 (52 %)	.395
Toxoplasma serology (pos)	22 (23 %)	36 (16 %)	.152
Weight (kg)	57 (50–107)	70 (19–180)	.654
Height (cm)	170 (128–198)	169 (70–195)	.101
BMI	23.6 (9.2–68.9)	24.2 (6.2–34.6)	.095
Male	48 (50 %)	107 (47 %)	.392
Age (years)	51 (10–70)	46 (7–75)	**.035**

Data are mean (SD), number (%), or median (range). The numbers are based on patients with data available.
COPD: chronic obstructive pulmonary disease; AAT1: Alpha 1-antitrypsin deficiency; PH: pulmonary hypertension; CF: cystic fibrosis; PF: pulmonary fibrosis; CMV: cytomegalovirus; EBV; Epstein-barr virus; BMI: body-mass index; FVC: forced volume vital capacity; FEV1; forced volume expiratory capacity 1 sec; 6MWT: 6-min walking test; AST: aspartate transaminase; ALT: alanine transaminase; SLTx: single-lung transplantation; DLTx: double-lung transplantation; HLTx: heart-lung transplantation; ReLTx: re-lungtransplantation; ATG: anti-thymocyte globulin; ECMO: extracorporeal membrane oxygenation.
Significant values are shown as bold.

Table 16. Cause of death after lung transplantation stratified between groups of allograft ischemic time in minutes (≤120, 121–240, 241–360 and 361+).

	Allograft ischemic time (min)				
Total N	≤120 ($n = 3$)	121–240 ($n = 35$)	241–360 ($n = 69$)	361+ ($n = 44$)	p Value
Cause of death					.807
Rejection	1 (33 %)	8 (23 %)	22 (32 %)	11 (25 %)	
Infection	2 (67 %)	10 (28.5 %)	15 (22 %)	16 (37 %)	
Malignancy	0 (0 %)	7 (20 %)	8 (11 %)	5 (11 %)	
Miscellaneous	0 (0 %)	10 (28.5 %)	24 (35 %)	12 (27 %)	

The group called "miscellaneous" is defined as patients with mortality caused by myocardial and cerebral ischaemia, and multiple organ failure such as renal and liver in addition to other causes related to the patient's old age and individual health status.

Survival assessment

Survival up to 1-year

Cumulative re-transplantation-free survival estimates in patients with a limited survival up to 1 year are illustrated in terms of percentage with an upper/lower 95% CI:

Ischaemic time ≤ 120 minutes showed 100-day and 200-day survival rates of 100%. Ischaemic time groups of 121-240, 241-360 and 361+ minutes had 100-day and 200-day survival rates at the equivalent time intervals of **94%** (CI: 88–99), **92%** (CI: 85–99); **93%** (CI: 89–97), **89%** (CI: 84–95); **92%** (CI: 86–98) and **91%** (CI: 84–97), respectively ($p<0.05$).

There were significant differences in survival rate estimates between ischaemic time ≤ 120 minutes versus 360+ minutes ($p<0.05$).

Survival up to 5 years

Pairwise comparisons between the groups showed significant differences in survival estimates between ischaemic time ≤ 120 minutes versus 241-360 minutes, ischaemic time 121-240 minutes versus 241-360 minutes ($p<0.05$).

Survival up to 10 years

Pairwise comparisons between the groups showed significant differences in survival estimates between ischaemic time ≤ 120 minutes versus 241-360 minutes ($p<0.05$).

Survival up to 15 years

No significant differences were found for patients who underwent lung transplantation between 1990 and 2005 ($p>0.05$) *(Figures 33-34).*

Survival in emphysema patients

No significant pairwise comparisons were found in these patients with a limited survival up to 5 years and transplanted overall between 1990-2016 *(Figure 35).*

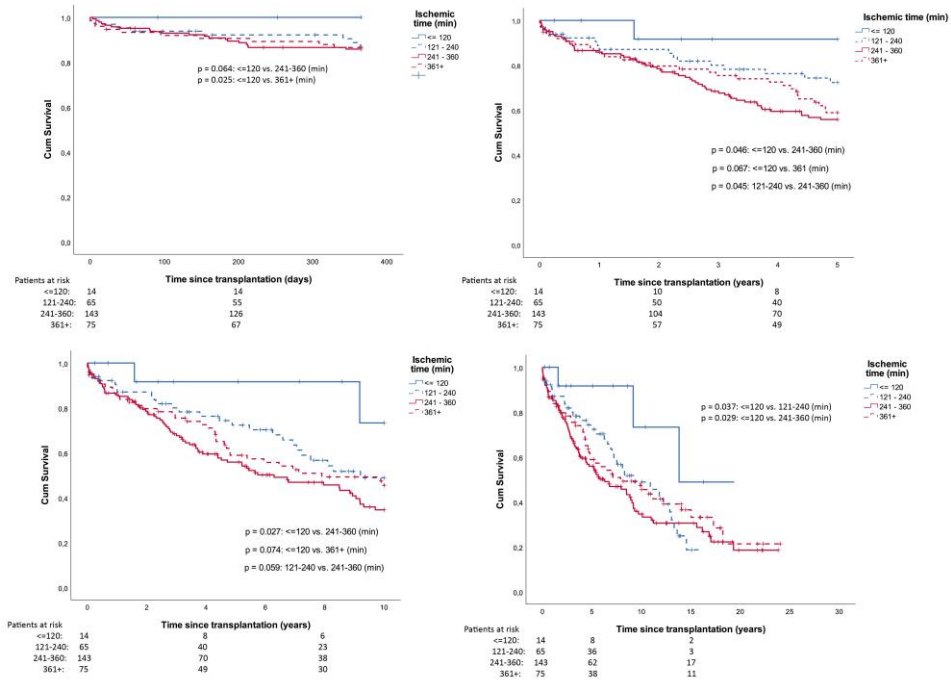


Fig. 33. Cumulative retransplantation-free survival for allograft ischemic time groups in minutes (≤120, 121–240, 241–360, 361+) for patients with a limited survival up to 1-year (top left), 5 years (top right), 10 years (bottom left) and overall for transplants between 1990 and 2016 (bottom right).

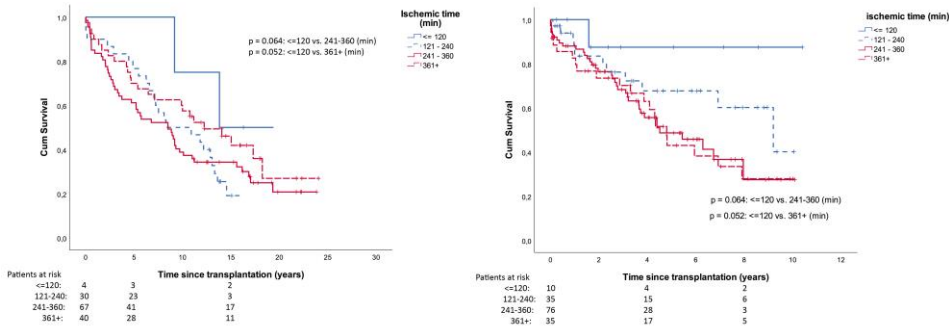


Fig. 34. Cumulative retransplantation-free survival for allograft ischaemic time groups in minutes (≤120, 121–240, 241–360, 361+) for transplants occurring in the periods 1990–2005 (left). 2006–2016 (right).

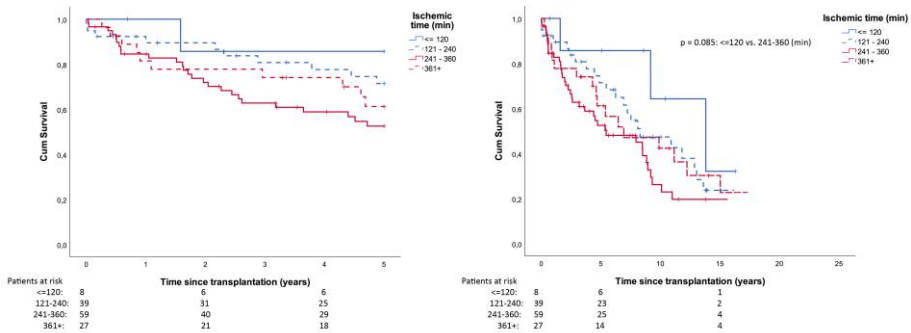


Fig. 35. Cumulative retransplantation-free survival for allograft ischaemic time groups in minutes (≤120, 121–240, 241–360, 361+) in emphysema patients (COPD þAAT1) with a limited survival up to 5 years (left) and in emphysema patients transplanted overall between 1990 and 2016 (right). COPD: chronic obstructive pulmonary disease; AAT1: Alpha 1-anitrypsin deficiency.

Cox regression (overall patients)

The Cox proportional hazards model evaluating ischaemic time (hours) and other recipient/donor risk factors are shown (*Table 17).*

In the multivariable analysis adjusting for *Tx-year, Tx-type, and recipient-age*; IT showed a hazard ratio (HR) of 1.125 (1.024-1.235) ($p<0.05$).

In the univariable analysis for IT interacting with recipient and donor age, respectively, an HR of 1.002 (1.001-1.003) was shown ($p<0.05$) in addition to IT interacting with recipient BMI with a HR of 1.004 (1.001-1.008) ($p<0.05$).

Cox regression (emphysema patients)

Cox regression analyses regarding cumulative incidence of death up to 5 years among emphysema patients (COPD+AAT1) are shown (*Table 18).*

In the multivariable analysis concerning ischaemic time (hours) for overall lung transplantation and in emphysema patients, an HR of 1.073 (1.001-1.151) and an HR of 1.125 (1.011-1.251) was shown, respectively ($p<0.05$).

In the univariable analysis for ischaemic time (hours) interacting with recipient age, the overall patients with a limited survival up to 5 years had an HR of 1.001 (1.000-1.002) whilst emphysema patients with a limited survival up to 5 years had an HR of 1.002 (1.001-1.004) ($p<0.05$).

Table 17. Cox proportional hazards model evaluating allograft ischemic time (IT) in hours and additional recipient/donor risk factors for limited survival up to 1-, 10-year and overall survival between 1990 and 2016.

	1-year			10-year			Overall		
	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Univariable									
IT (h)	1.119	1.017–1.230	**.021**	1.014	0.941–1.091	.720	1.000	0.930–1.076	.997
ATG	1.383	0.644–2.968	.404	1.304	0.915–1.860	.142	1.306	0.942–1.812	.110
Recipient-BMI	1.035	0.958–1.118	.384	1.035	0.995–1.078	.088	1.039	1.001–1.078	**.047**
Donor-BMI	0.992	0.951–1.036	.722	0.998	0.987–1.009	.716	0.997	0.985–1.009	.616
Recipient-male	1.554	0.816–2.960	.180	1.210	0.870–1.683	.257	1.148	0.848–1.566	.372
Donor-male	0.840	0.444–1.587	.590	1.055	0.759–1.467	.748	1.117	0.824–1.513	.477
Donor-age	1.000	0.981–1.020	.996	1.005	0.995–1.016	.316	1.007	0.997–1.017	.176
Recipient-age*IT (h)	1.002	1.001–1.003	**.002**	1.001	1.000–1.002	.098	1.001	1.000–1.002	.084
Recipient-BMI*IT (h)	1.004	1.001–1.008	**.016**	1.000	1.000–1.000	.386	1.001	0.998–1.004	.457
Donor-age*IT (h)	1.002	1.000–1.003	.059	1.001	0.999–1.002	.352	1.001	0.999–1.002	.325
Donor-BMI*IT (h)	1.001	0.999–1.003	.547	1.000	0.998–1.002	.806	0.999	0.997–1.001	.594
Multivariable									
[a]IT (h)	1.125	1.024–1.235	**.014**	1.035	0.966–1.109	.325	1.027	0.960–1.099	.442

[a]Adjusted for recipient age, Tx-type and Tx-year (before vs. after 2005).
ATG: Anti-thymocyte globulin; CI: confidence interval; HR: hazard ratio.
Significant values are shown as bold.

Table 18. Cox proportional hazards model evaluating allograft ischemic time (IT) in hours and additional recipient/donor risk factors for 5-year survival: for all patients and in emphysema-patients respectively.

	5-year			5-year emphysema-patients		
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Univariable						
IT (h)	1.063	0.987–1.144	.104	1.108	0.994–1.235	.065
ATG	1.136	0.751–1.717	.546	1.565	0.800–3.060	.191
Recipient-BMI	1.027	0.980–1.077	.262	1.033	0.959–1–113	.387
Donor-BMI	0.999	0.990–1.008	.821	0.996	0.977–1.014	.650
Recipient-male	1.256	0.855–1.846	.245	1.335	0.751–2.373	.325
Donor-male	1.029	0.700–1.511	.885	1.244	0.702–2.205	.455
Donor-age	1.000	0.988–1.012	.956	1.011	0.992–1.029	.265
Recipient-age*IT (h)	1.001	1.000–1.002	**.024**	1.002	1.001–1.004	**.004**
Recipient-BMI*IT (h)	1.002	1.000–1.005	.066	1.005	1.000–1.009	**.039**
Donor-age*IT (h)	1.001	1.000–1.002	.161	1.002	1.000–1.003	**.035**
Donor-BMI*IT (h)	1.000	0.999–1.002	.709	1.000	0.998–1.002	.925
Multivariable						
[a]IT (h)	1.073	1.001–1.151	**.047**	1.125	1.011–1.251	**.030**

[a]Adjusted for recipient age, Tx-type and Tx-year (before vs. after 2005).
ATG: Anti-thymocyte globulin; CI: confidence interval; HR: hazard ratio.
Significant values are shown as bold.

21.4 Paper IV

A total of 46 patients underwent double lung transplantation in our clinic at Lund University Hospital, Sweden. They were verified to have CLAD with phenotype BOS based on pulmonary function tests, chest imaging, and transbronchial biopsies. Plasma was collected and analysed for protein biomarkers using a multiplex immunoassay at baseline and at 1 year.

The plasma of patients was analysed for proteins using a high component, multiplex immunoassay that enables analysis of protein biomarkers. A total of 644 proteins in plasma were detected using the PEA, (*Figure 36*). Plasma samples were taken at baseline following double lung transplant. Of those 46 patients, 32 were analysed again after 1 year. Six patients were excluded due to re-transplantation secondary to BOS, another five died, and three were lost to follow up.

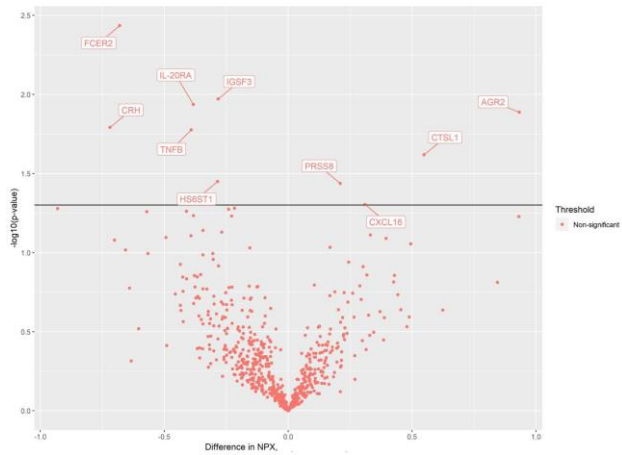


Fig. 36. A volcano plot of the 644 proteins analysed using the PEA. A linear regression model compared the two groups with the solid line indicating a p value of 0.05.

Proteins on the positive x-axis have higher NPX values in the BOS grade 1–3 group, and proteins on the negative x-axis have higher NPX values in the BOS grade 0 group.

PEA Proteomic analysis

Comparing BOS grade 0 to BOS grade 1–3. Comparison of BOS grade 0 to grades 1–3 showed significant differences in plasma levels of CRH, low affinity immunoglobulin epsilon Fc receptor (FCER2), interleukin-20 receptor subunit alpha (IL-20RA), TNF-β (TNFB), and immunoglobulin superfamily member 3 (IGSF3).

These proteins were significantly lower in patients who developed BOS *(Figure 37).*

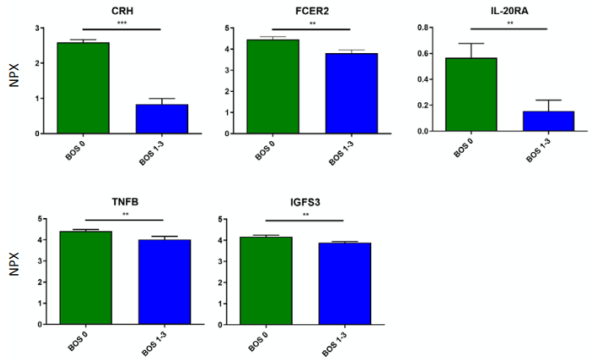


Fig. 37. Mean and SEM of five of the most significant proteins. These protein levels were all significantly lower among patients with BOS compared to those with grade 0.

** p<0.01, *** p<0.001.

CRH: corticotropin releasing hormone; FCER2: low affinity immunoglobulin epsilon Fc receptor; IL-20RA: Interleukin-20 receptor subunit alpha; TNFB: TNF-β; IGFS3: immunoglobulin superfamily member 3; BOS: bronchiolitis obliterans syndrome.

Comparing three groups: BOS grade 0 versus BOS grade 1 versus BOS grades 2–3 showed that CRH, IL-20RA, and FCER2 had significantly lower levels in patients who developed BOS grade 1 and in patients with BOS grades 2–3 compared to BOS grade 0 *(Figure 38)*.

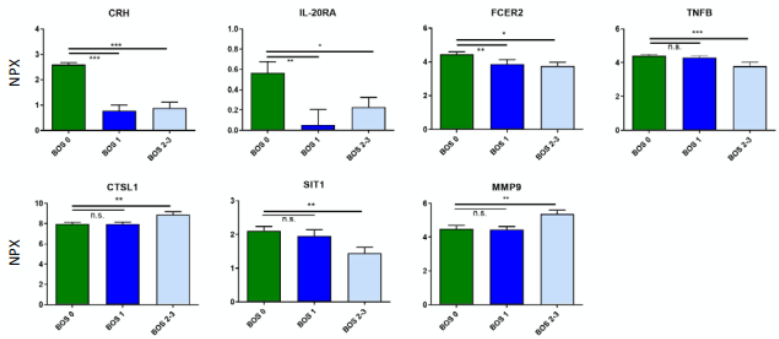


Fig. 38. Shows mean and SEM of seven of the most significant proteins. * p<0.05, ** p<0.01, *** p<0.001.

CRH: corticotropin releasing hormone; IL-20RA: Interleukin-20 receptor subunit alpha; FCER2: low affinity immunoglobulin epsilon Fc receptor; TNFB: TNF-β; CTSL1: cathepsin L1; SIT1: signalling threshold-regulating transmembrane adapter; MMP-9: matrix metalloproteinase 9; BOS: bronchiolitis obliterans syndrome.

In order to validate and confirm the PEA results, CRH and MMP-9 in plasma were measured by ELISA technology using separate methodology. Sensitivities of the CRH and MMP9 assays were 4.9 pg/mL and 10 pg/mL, respectively.

The baseline samples in 46 patients were also compared to the 32 patients who could be sampled at the 1-year follow-up *(Figure 39).*

MMP-9

At baseline, MMP-9 was significantly higher in those with BOS relative to those without. MMP-9 was significantly higher in grades 2–3 compared to either grade 1 or 0.

After 1 year, there was no significant difference between BOS grade 0 and BOS grades 1–3, although there was a trend towards increased MMP-9 levels.

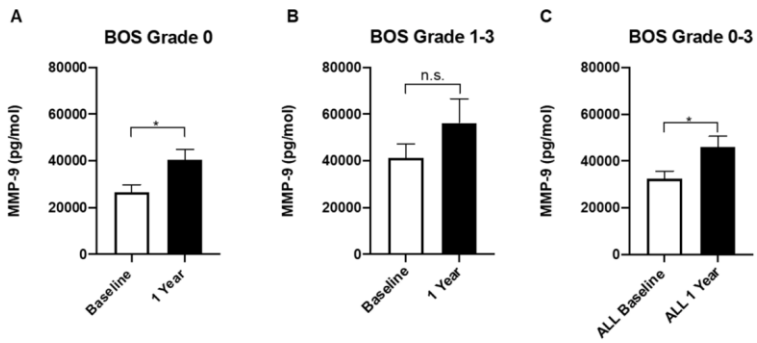


Fig. 39. Elevation of MMP within BOS groups. Following patients from baseline to 1 year, MMP-9 levels in plasma increased within grades of BOS. This increase was statistically signifcant in BOS grade 0 (A) and across all BOS grades 0–3 grouped together (C). When BOS grades 1–3 were examined (B).

*p<0.05.

CRH

At baseline, CRH was significantly lower in BOS grades 1–3 compared to grade 0. Only grade 1 showed a significant decrease in CRH plasma concentration compared to BOS grade 0. At the 1-year follow-up, CRH remained significantly decreased relative to grade 0 *(Figure 40).*

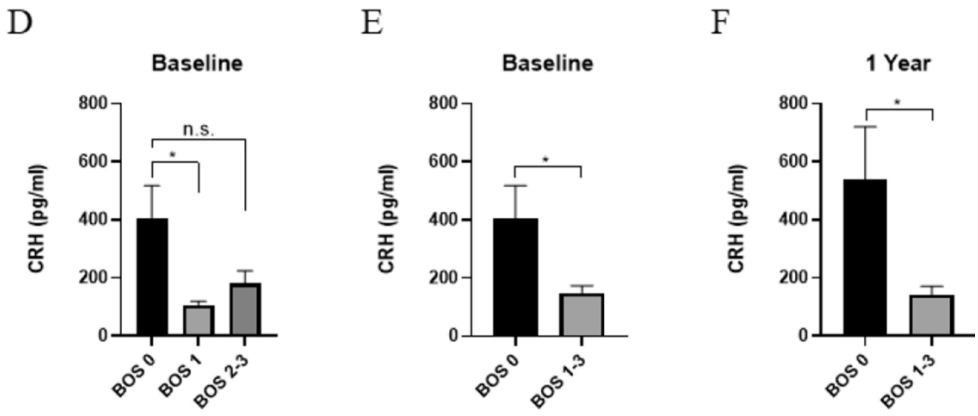


Fig. 40. CRH levels were lower in BOS patients compared to grade 0, both at baseline and at the 1-year followup.

*p<0.05.

When examining patients who remained at BOS grade 0 from the baseline time point to the 1-year follow-up, there was no significant change in their CRH levels. However, in patients whose BOS grade had increased between these two time points, there was a significant decrease in CRH *(Figure 41).*

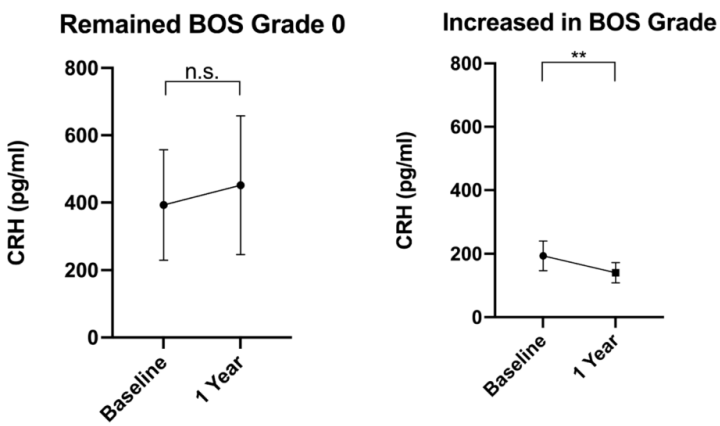


Fig. 41. CRH levels tracked through patient grade changes. In comparing BOS grade 0 patients who maintained their status at the 1-year followup, later CRH levels are noted to not be statistically different from their baseline plasma concentrations (A). In patients who increased BOS grades after 1 year (B), CRH plasma levels were statistically lower in their second sample.

**p<0.01. CRH: corticotropin releasing hormone; BOS: bronchiolitis obliterans syndrome.

21.5 Paper V

Establishment of lung injury using gastric content in the donor

Lung injury was established using gastric content in the donor and the animals were monitored continuously for haemodynamic parameters, ventilatory mechanical settings, blood gases and chest X-rays throughout the induction of lung injury. All donors developed infiltration seen on thoracic imaging after established lung injury *(Figures 42a,b)*. Histology of all donor and recipient lungs across time points are presented (*Figure 46*).

All donors showed overall haemodynamic stability and only required low dosages of inotropic support after gastric content administration (*Table 19*). The ratio of PaO_2/FiO_2 decreased significantly during the course of lung injury establishment from 527.4 ± 42.8 mmHg to 213.7 ± 134.7 mmHg (p=0.0005) *(Figure 43a)*.

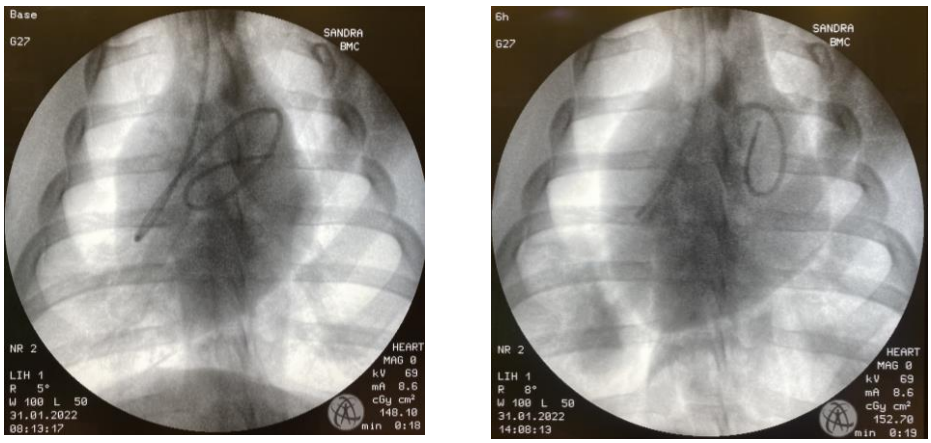


Fig. 42 a,b. Chest X-rays during establishment of lung injury. Lung injury was induced in the donors using gastric content, equally divided throughout the lung lobes bilaterally using a bronchoscopy. Afterwards, all donor pigs enrolled were kept under anaesthesia for 6 hours for the establishment of lung injury. A. (left) The figure demonstrates an example of the donor's chest X-ray before inducing lung injury. B. (right) The figure demonstrates an example of the donor's chest X-ray 6 hours after exposure to gastric content. Before administration of gastric content, the donors did not have infiltrations. The chest X-ray taken 6 hours later shows infiltration, especially in the lower lobe, and thus indicates established lung injury. (*PA catheter repositioned in this case)*

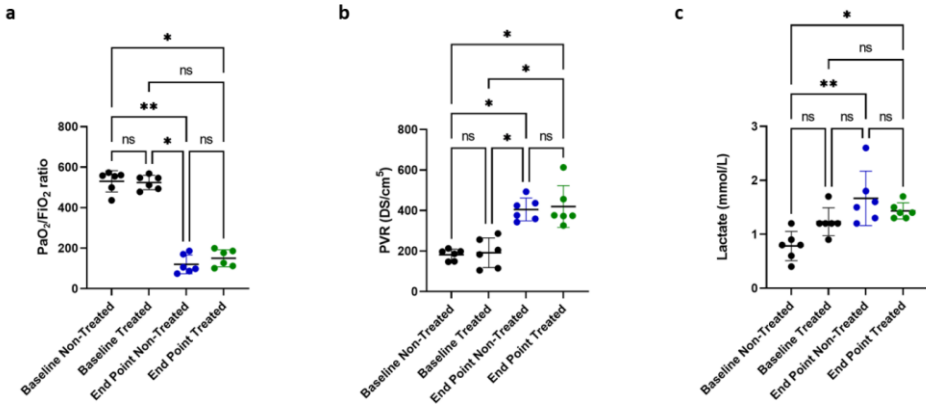


Fig. 43. Establishment of lung injury in the donor. Gastric content was used to induce lung injury, followed by 6 hours of observation while the donors were kept under anaesthesia. **a**. Comparison of the ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) between baseline and post-confirmation of injury, pre-treatment. **b**. Comparison of the pulmonary vascular resistance (PVR) between baseline and post-confirmation of injury, pre-treatment. **c**. Comparison of lactate between baseline and post-confirmation of injury, pre-treatment. Statistical differences were calculated by using the Student's t-test. The Mann–Whitney U-test or Wilcoxon test were used when data not distributed normally. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, p>0.05, ns. All values are represented as mean ± standard deviation. All pigs were included in the statistical calculations (n = 12).

No significant difference was observed in the ratio of PaO_2/FiO_2 between the donors assigned to the non-treated group compared to those in the treatment group, 6 hours after gastric content administration (p=0.1797) *(Figure 43a).*

Pulmonary vascular resistance significantly increased from 186.7 ± 52.9 DS/cm^5 at baseline to 412.4 ± 79.9 DS/cm^5 after established lung injury (p=0.0005) *(Figure 43b).*

No significant differences between the groups' PVR were observed after established lung injury (p=0.9654) *(Figure 43b).*

In a similar manner, lactate significantly increased from 1.1 ± 0.3 mmol/L to 1.5 ± 0.5 14 mmol/L during the establishment of lung injury (p=0.0005) *(Figure 43c).* When lactate was compared between the groups after established lung injury, no significant difference was found (p=0.9827) *(Figure 43c).*

Table 19: Overview recorded measurements in donor. Parameters measured during establishment of lung injury including the treated group (non-bold, n=6) or with non-treated (bold, n=6): oxygen saturation (SpO2, %), heart rate (HR, beats per minute, bpm), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), central venous pressure (CVP, mmHg); temperature (temp, °C), systolic pulmonary pressure (SPAP, mmHg), diastolic pulmonary pressure (DPAP, mmHg), mean pulmonary pressure (MPP, mmHg), pulmonary artery wedge pressure (PAPWP, mmHg), cardiac output (CO, L/min), cardiac index (CI, L/min/m2), systemic vascular resistance (SVR, dynes s/cm5), systemic vascular resistance index (SVRI, dynes s/cm5 m2), pulmonary vascular resistance index (PVRI, dynes s/cm5 m2), mechanical ventilator settings with volume-controlled ventilation: minute volume (MV, L/min), peak inspiratory pressure (PIP, cmH2O), peak end expiratory pressure (PEEP, cmH2O), tidal volume (Vt, mL), respiratory rate (RR, breaths/min), pH, partial pressure of carbon dioxides (PaCO2, mmHg), hemoglobin (Hb, g/L), lactate (mmol/L), base excess (mmol/L), dobutamine (µg/kg/min), noradrenaline (µg/kg/min).

	Baseline		**Endpoint / Confirmed Lung Injury**	
	Non-Treated	**Treated**	**Non-Treated**	**Treated**
Sat (%)	**99.2± 1.0**	98.7 ± 1.2	**94.0±4.4**	95.7±2.7
HR (bpm)	**85.2±13.7**	75.5±12.5	**81.8±15.4**	88.2±13.7
SBP (mmHg)	**108.5±15.4**	114.5±12.6	**116.7±14.7**	113.8±8.7
DBP (mmHg)	**69.8±13.2**	70.5±7.7	**87.7±9.4**	88.0±5.0
MAP (mmHg)	**84.2±11.5**	83.2±7.2	**100.7±9.5**	101.2±6.4
CVP (mmHg)	**5.3±1.0**	3.8±1.7	**6.5±1.8**	5.0±2.1
Temp (°C)	**37.0±0.6**	36.8±0.5	**38.1±1.0**	37.1±0.6
SPAP (mmHg)	**21.0±2.4**	22.8±3.5	**35.3±6.9**	33.0±3.4
DPAP (mmHg)	**11.8±2.2**	10.3±2.8	**18.3±5.5**	20.5±5.2
MPP (mmHg)	**15.8±2.6**	16.0±2.2	**24.8±6.0**	25.8±4.4
PAPWP (mmHg)	**8.0±2.8**	6.8±3.7	**10.2±2.6**	10.8±2.9
CI (L/min/m²)	**3.5±0.9**	3.7±0.8	**2.6±0.4**	2.5±0.4
SVR (DS/cm⁵)	**1582.7±559.5**	1582.5±408.7	**2374.5±317.5**	2611.3±389.3
SVRI (dynes s/cm⁵ m²)	**1947.7±694.0**	1867.0±495.6	**2956.2±533.5**	3134.2±490.4
PVRI (dynes s/cm⁵ m²)	**202.8±48.2**	223.5±76.0	**506.2±101.5**	493.5±112.0
MV (L/min)	**6.3±1.0**	5.4±0.7	**7.2±1.7**	6.3±1.6
PIP (cmH_2O)	**16.8±1.5**	16.1±0.7	**23.8±5.0**	21.8±3.9
PEEP (cmH_2O)	**5.2±0.4**	5.0±0.0	**7.3±3.9**	5.0±0
V_t (mL)	**278.8±30.4**	272.7±23.3	**273.2±38.9**	277.5±40.7
RR (breaths/min)	**21.5±2.1**	20.0±2.0	**26.0±4.9**	22.8±3.9
pH	**7.4±0.0**	7.4±0.0	**7.4±0.1**	7.4±0.0
$PaCO_2$ (mmHg)	**45.7±3.7**	44.2±4.5	**54.0±5.2**	49.5±3.0
Hb (g/L)	**101.8±12.7**	106.8±9.4	**108.5±18.5**	110.2±10.9
Lactate (mmol/L)	**1.0±0.3**	1.1±0.2	**1.5±0.7**	1.4±0.2
BE (mmol/L)	**7.2±1.9**	5.3±2.3	**4.7±4.4**	3.9±1.3
Dobutamine (µg/kg/min)	**0.0±0.1**	0.0±0.1	**0.2±0.2**	0.2±0.4
Noradrenaline (µg/kg/min)	**0.0±0.0**	0.0±0.0	**0.0±0.0**	0.0±0.0

Ex vivo lung perfusion with MSCs or placebo

Following lung injury, the lungs were harvested *en bloc*. Haemodynamic parameters, ventilatory settings, and blood gases were monitored continuously throughout the EVLP. An overview of the PaO_2/FiO_2 ratio and PVR during EVLP is presented (*Figures 44a,b).*

Over the course of EVLP, there was a significant increase in the ratio of PaO_2/FiO_2 in the pulmonary grafts in the treated group from 178.6 ± 36.9 mmHg to 374.4 ± 95.8 mmHg (p=0.0087, Figure 43a). The untreated lungs increased in the ratio of PaO_2/FiO_2 from 186.3 ± 18.0 mmHg to 198.3 ± 93.1 mmHg during EVLP, without any significant change over time (p>0.0999, *Figure 44a*). Overall, there was no significant difference between the treated and non-treated groups after 4 hours of EVLP (p=0.1829). Four out of six pigs within the treated group increased their PaO_2/FiO_2 ratio and met the criteria of being reaccepted into the donor pool after 4 hours of EVLP treatment while none of the lungs within the non-treated group met the thresholds for being utilised for transplantation. This represents a significant difference (p=0.0143) *(Figure 44d).*

PVR within the treated group was unchanged during the 4 hours of EVLP from 846.0 ± 575.3 DS/cm^5 to 551.9 ± 70.1 DS/cm^5 (p > 0.9999). The untreated group followed the same pattern with PVR at start of EVLP at 582.0 ± 233.8 DS/cm^5 and at the end of EVLP at 564.8 ± 302.5 DS/cm^5 (p > 0.9999). Comparing the groups, no significant difference was found after 4 hours of EVLP (p>0.9999) *(Figure 44b).*

Regarding the dynamic compliance of the lungs, there were no significant changes with respect to the groups at the end of EVLP compared to their baselines or between the two groups compared to each other (p>0.9999, *Figure 44c*).

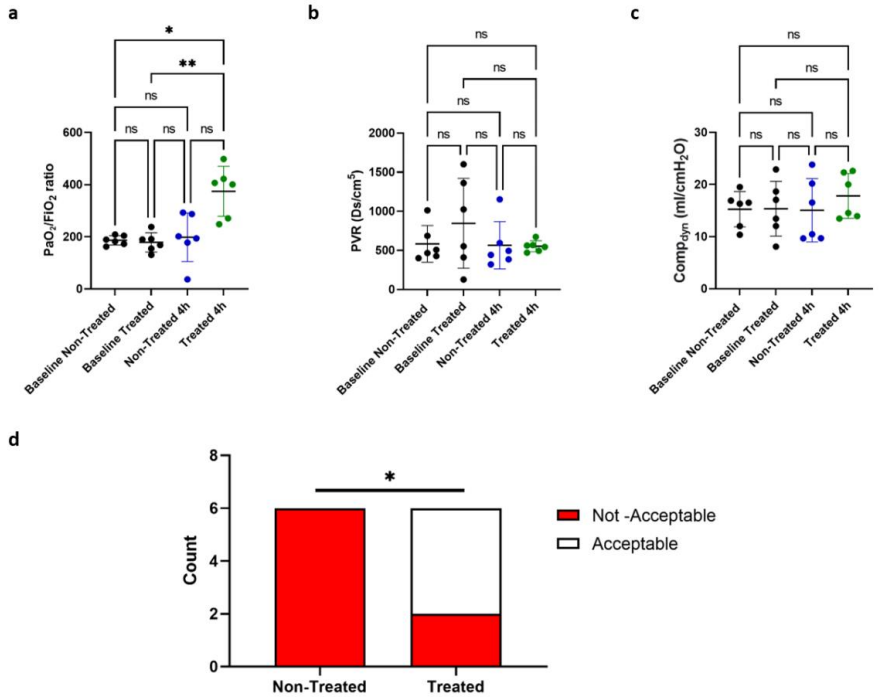


Fig. 44. *Ex vivo* lung perfusion with MSCs or placebo. After the lungs had been harvested, the pulmonary grafts were connected to *ex vivo* lung perfusion (EVLP) for 4 hours. The treatment group received EVLP with mesenchymal stromal cell (MSC) treatment and the non-treated group EVLP with placebo. **a**. Ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2), **b**. pulmonary vascular resistance (PVR) and **c.** dynamic compliance for treated and non-treated groups comparing values at baseline to values after 4 hours of EVLP. **d**. Comparison between groups regarding acceptable or not acceptable blood gases for transplantation after 4 hours of EVLP.

Statistical differences were calculated by using the Student's t-test or ANOVA. The Mann–Whitney U-test, Wilcoxon, or Friedman's test were used when the data were not distributed normally. A Chi-squared test was performed to analyse observed frequencies of categorical variables. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001, p>0.05, ns. All values are represented as mean ± standard deviation. All pigs were included in the statistical calculations (n = 12).

Follow-up of lung transplantation 0-60 hours

Haemodynamic parameters and pulmonary function were followed from the time of lung transplantation through 60 hours of follow-up and are shown (*Table 20*).

At the conclusion of the post-lung transplant monitoring period, the left transplanted lung alone was assessed via a right pneumonectomy performed at 68-72 hours. The transplanted lung was evaluated for haemodynamic parameters and pulmonary function (*Table 21*).

The treated group was found to have a higher PaO_2/FiO_2 ratio compared to the non-treated group, with treated recipients reaching a ratio of 433.4 ± 53.8 mmHg at 72

hours, and the non-treated group reaching 202.7 ± 114.1mmHg (p=0.0027, *Figure 45a*). At 72 hours, the treated group had a PVR of 232.0 ± 69.9 DS/cm^5, which was statistically significantly different compared to the non-treated group, which had a PVR of 414.2 ± 198.5 DS/cm^5 (p=0.0411, *Figure 45b*). There was no significant difference seen in lactate between the groups at 72 hours' post-lung transplant (p=0.4545) or in the dynamic compliance between the two groups (p>0.9999, *Figure 45c*).

PGD was assessed at 72 hours. In the non-treated group, two recipients developed PGD grade 3, three developed PGD grade 2 and one had PGD grade 0. None of the treated recipients developed PGD. This represented a significant difference between the groups (p=0.0138, *Figure 45d*).

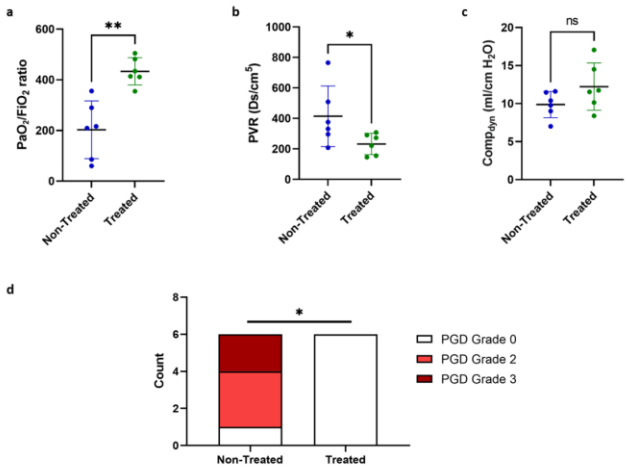


Fig. 45. Evaluation of pulmonary function and primary graft dysfunction at 72 hours. The recipients were kept under anaesthesia and monitored for 72 hours after lung transplantation. The last phase of the experiment consisted of a right pneumectomy followed by 4 hours' evaluation of the single transplanted lung. **a**. Ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2), **b**. pulmonary vascular resistance (PVR) and, **c.** dynamic compliance.**d**. development of PGD between the groups.
Statistical differences were calculated by using the Student's t-test. The Mann–Whitney U-test or Wilcoxon test were used when the data were not distributed normally. A Chi-squared test was performed to analyse observed frequencies of categorical variables. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001, p>0.05, ns. All values are represented as mean ± standard deviation. All pigs were included in the statistical calculations (n = 12).

Table 20: Overview measured parameters during 0-60 h follow-up. Clinical measurements in the post-transplantation follow-up including the treated group (non-bold, n=6) or with non-treated (bold, n=6)

	Baseline	1h	12h	24h	36h	48h	60h
Sat (%)	97.5±1.4	97.5±0.5	97.7±1.2	98.0±0.9	97.7±1.4	98.0±1.7	98.0±1.2
	99.3±0.5	**97.8±1.2**	**98.0±1.3**	**97.8±1.2**	**97.8±1.0**	**97.8±0.4**	**98.2±1.2**
HR (bpm)	82.0±17.7	74.5±7.5	78.8±19.5	76.2±10.6	78.5±10.8	77.0±18.1	72.4±13.9
	85.7±18.1	**77.8±9.4**	**76.2±11.8**	**84.5±17.0**	**78.3±4.8**	**74.5±9.0**	**76.7±9.8**
SBP (mmHg)	117.3±12.6	112.3±9.0	108.2±11.5	107.0±6.8	109.8±4.6	108.3±8.8	112.8±13.3
	110.2±23.8	**114.5±9.1**	**112.8±8.4**	**106.7±6.2**	**113.8±7.0**	**116.5±14.4**	**118.2±10.7**
DBP (mmHg)	74.8±19.1	76.7±6.9	70.3±10.5	71.0±13.0	78.2±14.6	78.5±11.6	76.4±13.5
	73.0±14.9	**76.7±5.5**	**74.3±7.3**	**68.0±5.7**	**80.0±10.5**	**81.3±12.7**	**80.0±12.5**
MAP (mmHg)	88.8±17.5	94.3±5.2	89.2±9.5	89.0±10.5	95.7±11.0	95.8±9.7	94.0±12.0
	88.5±18.2	**94.7±8.4**	**92.7±7.3**	**86.2±7.3**	**96.2±7.5**	**100.5±17.8**	**99.7±11.7**
CVP (mmHg)	7.2±1.5	4.8±2.8	6.7±2.5	5.0±3.1	7.2±2.8	6.2±2.9	7.0±1.8
	6.7±3.3	**7.7±2.5**	**6.7±2.3**	**5.8±1.2**	**7.2±1.5**	**8.0±4.5**	**5.7±0.8**
Temp (°C)	37.1±0.7	38.0±2.9	38.6±0.1	39.8±1.3	39.8±0.8	40.5±1.3	40.6±1.1
	37.6±0.8	**39.4±0.7**	**39.4±0.2**	**39.5±0.1**	**40.0±0.6**	**38.0±1.2**	**39.6±0.4**
SPAP (mmHg)	23.8±3.4	30.3±3.8	26.2±7.0	25.3±8.8	27.2±7.4	29.8±6.0	27.6±6.7
	21.5±2.1	**33.8±3.4**	**26.3±5.6**	**29.3±4.6**	**29.6±5.0**	**28.0±6.4**	**28.3±6.9**
DPAP (mmHg)	14.5±3.9	16.2±3.1	14.0±4.4	14.2±2.9	15.5±3.8	12.8±4.7	13.4±5.2
	12.7±2.9	**18.3±3.4**	**13.2±4.6**	**15.2±3.4**	**12.4±3.4**	**14.0±4.0**	**15.8±3.1**
MPP (mmHg)	18.3±3.1	22.3±3.1	16.2±4.2	18.0±5.0	20.0±4.8	19.5±5.4	19.0±4.6
	16.3±2.4	**25.0±4.0**	**19.2±4.5**	**21.0±3.5**	**20.0±3.2**	**19.8±3.6**	**21±4.8**
Wedge (mmHg)	13.2±2.7	11.0±5.3	8.8±1.7	8.8±1.3	11.2±2.0	11.0±3.0	9.8±2.5
	8.8±3.4	**9.0±1.5**	**8.3±4.3**	**9.8±1.9**	**10.2±0.8**	**11.0±3.6**	**10.3±2.7**
CO (L/min)	4.2±1.3	3.6±0.6	4.3±0.8	5.0±1.1	4.5±0.9	4.7±0.8	4.0±0.5
	3.8±0.8	**4.0±1.0**	**4.8±0.7**	**5.2±1.2**	**4.3±0.5**	**4.1±0.8**	**4.4±0.5**
CI (L/min/m^2)	3.4±1.2	2.8±0.3	3.5±0.6	4.0±0.9	3.6±0.6	3.7±0.8	3.2±0.4
	3.0±0.6	**3.2±0.8**	**3.9±0.5**	**4.2±1.0**	**3.5±0.4**	**3.2±0.4**	**3.6±0.3**
SVR (DS/cm^5)	1887.5±446.0	1937.3±338.9	1510.0±533.7	1305.8±210.4	1704.2±484.9	1594.7±354.6	1755.0±313.1
	1880.8±498.6	**1844.3±330.9**	**1480.2±188.3**	**1398.3±248.9**	**1450.2±325.66**	**1450.0±59.4**	**1766.0±219.0**
PVR (DS/cm^5)	153.8±60.7	304.7±87.9	188.6±62.7	151.0±66.9	176.4±76.0	172.2±67.7	190.4±45.3
	162.7±43.4	**347.2±111.2**	**191.8±57.1**	**177.3±24.1**	**159.8±46.1**	**183.5±67.2**	**198.8±74.3**
SVRI(dynes s/cm^5 m^2)	2434.7±761.9	2468.7±367.9	1855.0±625.2	1815.5±415.7	2067.4±454.6	2034.8±547.1	2297.0±556.8
	2321.7±558.0	**2296.3±454.8**	**1839.3±248.6**	**1662.3±281.9**	**1774.7±328.0**	**2203.0±14.1**	**2167.7±301.3**

PVRI(dynes s/cm^5 m^2)	200.8±101.5	434.5±280.1	232.2±72.6	191.3±80.7	219.0±83.7	217.5±82.8	250.0±75.8
	200.7±47.5	**433.8±152.9**	**239.5±72.8**	**224.2±38.2**	**196.3±54.7**	**248.5±128.0**	**243.8±91.5**
MV (L/min)	6.2±0.7	7.4±0.6	8.0±1.1	7.8±1.3	7.7±1.3	8.1±1.4	8.2±1.4
	5.7±0.6	**7.2±0.9**	**7.5±0.8**	**7.6±0.5**	**7.6±0.6**	**7.7±0.8**	**7.8±0.7**
PIP (cmH_2O)	17.8±2.6	25.6±1.7	24.1±2.1	20.7±6.7	23.3±1.7	25.1±1.3	25.5±3.0
	17.3±1.4	**24.9±1.7**	**24.4±1.8**	**25.2±2.2**	**25.5±2.7**	**27.5±4.3**	**27.1±3.2**
PEEP (cmH_2O)	5.3±0.5	5.7±1.2	6.2±1.2	6.2±1.2	5.7±1.2	6.7±1.5	5.8±0.6
	5.0±0.0	**6.0±1.5**	**5.5±1.4**	**5.8±1.3**	**6.0±1.1**	**5.7±1.2**	**5.7±0.8**
Vt (mL)	299.3±38.1	282.8±26.6	297.8±23.1	279.2±10.7	286.8±17.6	288.0±15.2	303.4±29.1
	283.7±14.8	**269.7±16.3**	**270.3±15.4**	**269.2±17.4**	**271.5±11.5**	**271.0±17.4**	**266.8±22.7**
RR (breaths/min)	21.0±1.7	26.2±2.1	27.0±3.2	27.3±3.1	27.3±3.1	27.8±3.3	26.9±2.7
	20.5±2.8	**26.8±1.9**	**28.0±2.5**	**28.4±2.1**	**28.3±2.2**	**28.5±1.6**	**29.5±1.6**
FiO_2 (%)	0.5±0.0	0.7±0.1	0.5±0.1	1.0±0.1	0.4±0.0	1.0±0.1	1.0±0.0
	0.5±0.1	**0.6±0.1**	**0.5±0.0**	**0.9±0.1**	**0.5±0.1**	**0.9±0.2**	**1.0±0.0**
pH	7.5±0.1	7.4±0.0	7.4±0.1	7.4±0.0	7.5±0.0	7.4±0.0	7.5±0.0
	7.5±0.0	**7.4±0.0**	**7.4±0.0**	**7.4±0.0**	**7.4±0.0**	**7.4±0.0**	**7.5±0.0**
$PaCO_2$ (mmHg)	44.2±6.0	48.0±5.2	45.0±5.2	49.5±3.7	46.5±3.0	49.5±3.7	48.0±0.0
	44.2±5.2	**50.2±7.5**	**48.0±4.5**	**51.0±3.7**	**48.0±3.0**	**48.7±2.2**	**46.5±5.2**
PaO_2 (mmHg)	250.1±38.0	159.5±67.7	165.4±36.4	336.6±174.5	135.3±39.5	335.4±172.9	396.0±55.2
	266.6±38.7	**160.4±40.6**	**193.8±36.4**	**372.1±77.3**	**141.5±16.1**	**371.0±54.9**	**388.1±74.8**
Hb (g/L)	106.7±12.9	108.3±10.7	101.5±14.0	94.0±11.3	86.7±11.8	84.8±10.8	84.0±15.6
	111.5±9.2	**108.5±12.6**	**94.3±8.1**	**86.8±7.8**	**86.7±11.3**	**83.2±11.6**	**80.5±10.6**
Lactate (mmol/L)	1.2±0.3	1.1±0.4	1.1±0.9	0.8±0.7	0.5±0.1	0.5±0.1	0.5±0.0
	1.0±0.2	**2.0±2.1**	**0.8±0.6**	**0.5±0.1**	**0.5±0.1**	**0.6±0.2**	**0.4±0.1**
BE (mmol/L)	6.7±1.9	5.2±2.4	5.5±4.7	9.3±2.2	10.2±5.1	10.0±4.3	11.9±2.9
	7.4±1.1	**3.3±4.0**	**6.4±3.8**	**7.2±3.1**	**9.1±2.6**	**8.9±3.7**	**8.5±3.0**
PaO_2/FiO_2 (mmHg)	514.9±42.6	235.9±83.2	362.9±79.4	425.0±61.1	306.9±70.9	422.2±56.2	396.0±55.12
	599.2±58.2	**275.9±60.9**	**375.5±76.9**	**417.5±73.6**	**304.4±28.5**	**438.2±107.0**	**388.1±74.8**
Dobutamine (µg/kg/min)	0.0±0.1	0.2±0.2	0.8±1.2	0.5±0.5	0.4±0.4	0.5±0.0	0.2±0.2
	0.1±0.1	**0.3±0.2**	**0.3±0.2**	**0.3±0.2**	**0.2±0.2**	**0.3±0.5**	**0.3±0.5**
Noradrenaline (µg/kg/min)	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.0±0.0	0.1±0.1	0.0±0.0
	0.0±0.0	**0.1±0.1**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**

Table 21: Overview measured values during the 4 last hours. Clinical measurements in the last 4 hours post-pulmectomy including the treated group (non-bold, n=6) or with non-treated (bold, n=6): oxygen saturation (SpO2, %), heart rate (HR, beats per minute, bpm), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), central venous pressure (CVP, mmHg); temperature (temp, °C), systolic pulmonary pressure (SPAP, mmHg), diastolic pulmonary pressure (DPAP, mmHg), mean pulmonary pressure (MPP, mmHg), pulmonary artery wedge pressure (PAPWP, mmHg), cardiac output (CO, L/min), cardiac index (CI, L/min/m2), systemic vascular resistance (SVR, dynes s/cm5), systemic vascular resistance index (SVRI, dynes s/cm5 m2), pulmonary vascular resistance index (PVRI, dynes s/cm5 m2), mechanical ventilator settings with volume-controlled ventilation: minute volume (MV, L/min), peak inspiratory pressure (PIP, cmH2O), peak end expiratory pressure (PEEP, cmH2O), tidal volume (Vt, mL), respiratory rate (RR, breaths/min), pH, partial pressure of carbon dioxides (PaCO2, kPa), hemoglobin (Hb, g/L), lactate (mmol/L), base excess (mmol/L), dobutamine (µg/kg/min), noradrenaline (µg/kg/min).

	Before pulmectomy		**4h Post pulmectomy**	
	Non-Treated	**Treated**	**Non-Treated**	**Treated**
Sat (%)	**96.5±3.0**	98.5±1.0	**95.2±5.2**	98.7±1.0
HR (bpm)	**74.8±13.7**	75.2±9.9	**103.8±14.7**	84.7±4.2
SBP (mmHg)	**114.5±18.3**	119.5±11.5	**94.8±12.8**	102.0±6.1
DBP (mmHg)	**80.7±20.7**	82.7±11.6	**62.3±21.2**	70.2±9.5
MAP (mmHg)	**96.0±18.7**	100.3±11.3	**74.5±22.1**	85.2±6.7
CVP (mmHg)	**7.0±2.9**	6.5±1.4	**6.5±2.5**	7.8±1.9
Temp (°C)	**40.5±1.0**	39.6±0.4	**40.1±2.8**	40.2±0.4
SPAP (mmHg)	**28.3±6.4**	27.5±7.5	**37.3±5.5**	30.2±5.6
DPAP (mmHg)	**16.7±3.9**	15.5±4.5	**20.8±3.9**	15.7±5.5
MPP (mmHg)	**22.0±4.7**	20.8±5.2	**26.8±4.5**	21.3±4.8
PAPWP (mmHg)	**11.2±2.7**	9.8±2.0	**11.2±2.5**	10.3±2.0
CO (L/min)	**3.6±0.9**	4.4±0.6	**3.4±1.3**	3.7±0.7
CI (L/min/m^2)	**2.9±0.7**	3.6±0.4	**2.7±1.0**	3.0±0.6
SVR (DS/cm^5)	**1706.7±480.7**	1881.3±248.7	**1785.2±501.1**	1861.0±477.9
SVRI (dynes s/cm^5 m^2)	**2157.8±600.5**	2255.3±356.3	**2264.8±669.1**	2287.3±614.5
PVRI (dynes s/cm^5 m^2)	**342.3±177.6**	225.2±68.6	**510.5±209.5**	307.7±119.2
MV (L/min)	**7.7±1.6**	7.8±0.7	**8.7±1.1**	8.0±0.7
PIP (cmH_2O)	**26.2±2.6**	25.3±3.5	**34.7±5.1**	27.2±4.0
PEEP (cmH_2O)	**5.0±0.5**	5.7±0.8	**5.8±0.1**	5.5±0.2
V_t (mL)	**297.0±31.3**	266.5±20.9	**270.7±32.5**	257.5±31.7
RR (breaths/min)	**27.1±2.3**	29.3±1.4	**32.7±6.9**	31.1±4.5
pH	**7.5±0.0**	7.4±0.0	**7.3±0.1**	7.4±0.0
$PaCO_2$ (kPa)	**6.2±0.5**	6.3±0.6	**7.4±1.4**	5.9±0.3
Hb (g/L)	**83.0±9.5**	80.2±7.4	**85.2±18.0**	80.8±13.0
Lactate (mmol/L)	**0.7±0.2**	0.6±0.2	**1.3±1.2**	0.8±0.2
BE (mmol/L)	**9.0±4.4**	6.6±1.7	**4.0±5.2**	4.3±2.1
Dobutamine (µg/kg/min)	**0.3±0.2**	0.3±0.5	**0.8±0.3**	0.1±0.1
Noradrenaline (µg/kg/min)	**0.0±0.0**	0.0±0.0	**0.1±0.0**	0.0±0.0

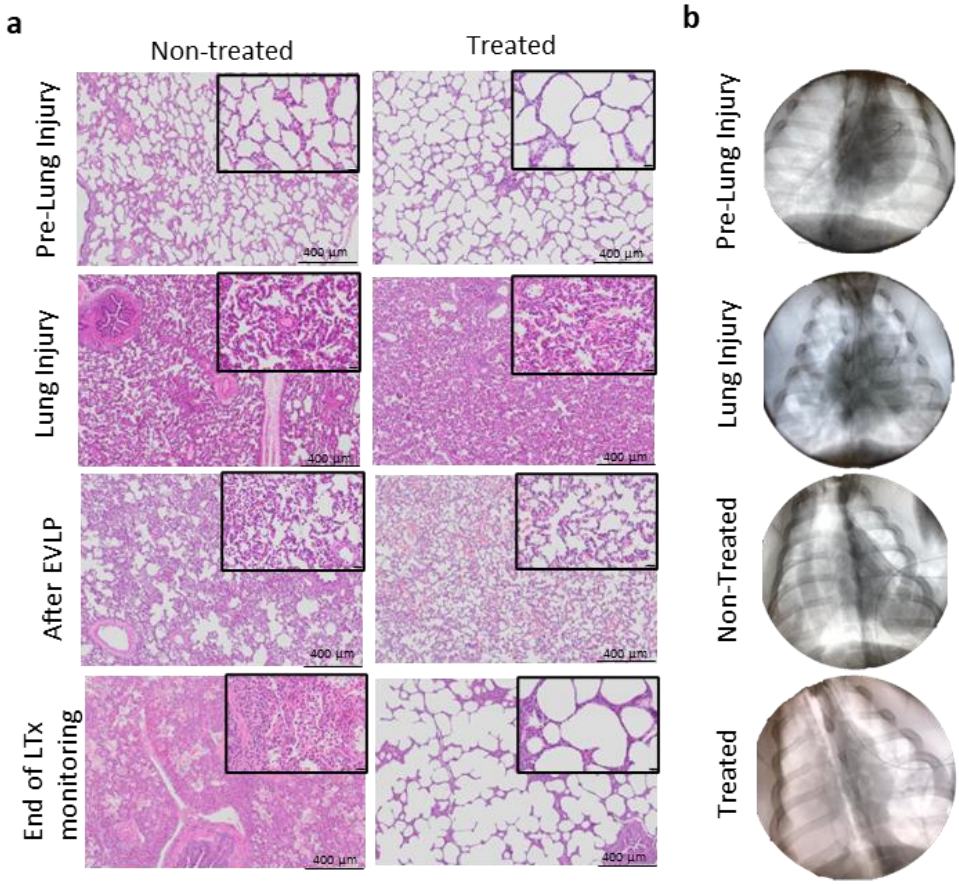


Fig. 46. Histopathological changes and X-rays during the course of the experiment. a. Images showing haematoxylin and eosin (H&E) histology of non-treated (left) lungs and treated (right) lungs pre-lung injury, at the end of lung injury induction, after EVLP and at the endpoint of the experiment. Scale bar in the larger image represents 400 µm. The callout shows a magnified portion of the tissue where the scale bar represents 20 µm. b. Representative X-ray images taken pre-lung injury, at the end of lung injury induction and at the endpoint of the experiment for non-treated and treated animals.

22 Discussion

22.1 Paper I

This study investigated the efficacy of a cytokine adsorber as a method in the treatment of ARDS-damaged lungs as well as to restore the lungs' acceptability for transplantation and reduce the incidence of development PGD in recipients. This model is an established model of ARDS, as published previously[214].

The porcine EVLP model provides an ideal preclinical platform, and the protocols adhere closely to human protocols in addition to the similarities to human anatomical and biological features.

Cytokine adsorption effect has recently been evaluated in combination with EVLP in preclinical settings and has helped in the reconditioning of healthy lungs from advanced cold ischaemic storage[101, 215].

To address this method, donor lungs with an LPS-induced ARDS injury were transplanted and treated with cytokine adsorption. LPS taken from the outer membrane of Gram-negative bacteria and given intravenously results in damage to the endothelial lining of the vessel walls of the lung to induce programmed cell death (apoptosis) which is suspected to be the underlying principle in sepsis pathogenesis[216].

This form of induced ARDS (LPS model) has been studied in large animal models in light of the clinical potential of the disease. Other forms of provoking ARDS, including repeated lavage model and oleic acid (fatty acid embolism model), smoke/burn, all these models result in lung pathology but they do not completely reproduce the pathophysiology of human ARDS[214].

An advantage of the use of the endotoxin model is that it has a pathophysiology similar to that of clinical ARDS. This provides an opportunity to explore the expansion of the donor pool, as many organs are rejected due to acute lung injury.

In contrast, the use of EVLP is an already confirmed method and alone can reduce the incidence of acute injury in the lungs. In combination with a cytokine adsorber, EVLP can treat healthy lungs subjected to extended cold ischaemic storage[101, 215].

Regarding the pathology induced in this study, all donors developed mild-to-moderate ARDS with significantly lower gas exchange capacities as measured by the PaO_2/FiO_2 ratio before lung harvest. This adheres to the Berlin definition of the syndrome[119, 120]. The diagnosis of ARDS was further confirmed histopathologically, by diffuse alveolar damage characterised by hyaline membranes lining the alveolar spaces in a distinctive manner[217].

Further evidence of the onset of ARDS was supplemented by the blinded scoring conducted. Graded samples were based on morphological changes, such as thickening of the alveolar walls, haemorrhage, and atelectasis in LPS-treated lungs as compared to controls.

The administration of LPS also caused a dramatic increase in early response cytokines, specifically IL-6, IL-8, IL-1β and TNFα in all donors. It has been reported previously that these proinflammatory cytokines have been suggested to be biomarkers of morbidity and mortality in ARDS, and TNFα has been recognised in the pathogenesis of ARDS[211, 218].

The use of extracorporeal haemoadsorption techniques to reduce proinflammatory cytokines and tissue damage has been explored within several associated surgical conditions. Such techniques have been used *in vivo* during human orthotopic heart transplantation and in human kidney transplantation[92, 98, 207], as well as in patients with severe sepsis and acute lung injury. The techniques have been reported to have reduced the levels of IL-6, IL-8, IL-1β and TNFα[92, 93, 219]. However, the method has not been examined in donator lungs damaged by ARDS which are then transplanted.

CytoSorb® is a cytokine adsorption filter that remove substances through polymer beads. These devices target middle and low molecular weight molecules, thus reducing levels of cytokines.

After established ARDS, harvested lungs were placed in cold storage in Perfadex® PLUS solution for 2 hours. Afterwards, they were connected to EVLP for 4 hours (mimicking clinical transplantation). Throughout EVLP, the cytokine adsorption-treated group had improved gas exchange capacity and most achieved a PaO_2/FiO_2 ratio above 300, a value that is regarded as being clinically acceptable for transplantation. In contrast, the PaO_2/FiO_2 ratios of non-treated lung did not reach acceptable levels for transplantation.

Furthermore, lungs in the treated group experienced significantly reduced BALF levels of IL-1β relative to the non-treated lungs. Other cytokines were also generally decreased throughout EVLP. This indicates a state of reduced inflammation when comparing the two conditions, further supported by the decreased number of

immune cells and atelectasis seen on histological examination in the treated lungs compared to the non-treated ones.

To investigate the functionality of the adsorption in this setting, the transplanted lung was monitored for 48 hours. Then, in order to specifically evaluate the just-transplanted lung function, a right-sided pneumonectomy was subsequently performed.

During the first 48 hours of post-transplantation monitoring, there was an obvious reduced need for inotropic support along with greater haemodynamic stability in both the one-step and two-step treatments. This is in agreement with the findings of studies of cytokine adsorption in patients with sepsis, in whom the treatment reduced the required dose of noradrenaline[220].

In this model, recipients were also found to have reduced cytokine levels and there were significant decreases in neutrophils and total white blood cells counts in the treated groups. Decreasing levels of cytokines are particularly important in ARDS given that clinical studies have shown increased levels of IL-6 and TNFα in the plasma and BALF samples of those who do not survive. Furthermore, there is a correlation of IL-6 with a longer time spend on ventilation[212].

Interestingly, in the first day post-transplantation, there was no difference in the gas exchange capacity between all groups. However, during the second day and especially after the right pneumonectomy, a significant difference in gas exchange could be seen between the two-step treatment and the non-treated group. The two-step-treated lungs performed better than the one-step ones concerning the ratio and the findings from the histological and apoptotic score points.

Additionally, using cytokine adsorption may have potentially minimised the risk of developing fatal septicaemia in the treated recipient. The need for less inotropic support and greater haemodynamic stability of treated recipients could also be attributed to reduced cytokine levels.

In our study, three recipients showed signs of septicaemia post-transplantation.

One recipient in the two-step treated group developed bacteraemia 8 hours after transplantation but recovered with no subsequent signs.

Another recipient in the non-treated group also developed fatal septicaemia and died 9 hours' post-transplantation despite advanced intensive care.

The third recipient in the one-step treated group developed mild septicaemia with a severe tachyarrhythmia. The recipient was treated with albumin, a magnesium infusion, potassium, and intravenous lidocaine without any positive effect. The

haemoadsorber was established and the tachyarrhythmia subsequently stabilised and resolved within 1 hour of haemoadsorption.

Interestingly, in the two-step treated group, one recipient developed dramatic pulmonary oedema after 2 hours of EVLP. Up to 1.2 L of fluid was drained from the trachea during EVLP, but at the end of the post-transplantation observational period, virtually all oedema had been resorbed and the graft showed excellent gas exchange capacity and no signs of PGD. Furthermore, the *wet–dry ratios* of the lung tissue when comparing the end of EVLP to the end of lung transplantation showed a decrease between these time points. This suggests that cytokine adsorption was of particular importance during haemoperfusion post- transplantation and that it reduces the accumulation of fluid in the tissue.

These incidences of oedema and septicaemia illustrate how the addition of a cytokine adsorber may support the restoration of traditionally non-acceptable donor lungs in the critical 3 days immediately following surgery. These days are crucial, given the mortality associated with PGD.

In this study, five of six two-step treated recipients and two of four one-step treated recipients had no PGD at all. In contrast, five of six non-treated recipients developed severe grade 3 PGD.

This additive effect of treatment in both EVLP and post-transplant (the two-step group) with respect to just post-transplant alone (one-step group) is clearly demonstrated in the comparison of the PaO_2/FiO_2 ratio at the end of the experiment. Not only did both groups improve relative to the non-treated recipients, but the two-step treated recipients had significantly higher scores than the one-step treated ones.

Additionally, leukocyte levels were significantly lower in the treated animals. This immunological suppression response afforded by a cytokine adsorber could be responsible for the reduced incidence of PGD.

There are some limitations to the present study. One concern with cytokine adsorption in general is the potential for adsorption and removal of non-desired targets, with a previous study finding that plasma drug levels may decrease with treatment[101]. When translating the findings of this study to a clinical setting, concern over potentially diminishing drug levels should result in careful measurement of the recipient's plasma levels and precautions taken to maintain therapeutic levels.

The intensive care required to sustain the pigs prohibits a longer follow-up over weeks or months, as might be desired to understand long-term outcomes, and thus the animals were only followed for 48 hours plus the time post-pneumonectomy. This timeline did not allow for investigation of what effect cytokine adsorption may have on acute rejection or on CLAD.

In consideration of the injury model used within this study, administration of LPS was chosen for its ability to reproduce an ARDS state, but its use is limited as it does not represent a multi-factorial lung injury seen in human donor lungs.

As double lung transplantation is not possible in pigs due to anatomical challenges on the right bronchus, a left lung transplant was conducted in this model.

22.2 Paper II

A major challenge in clinical lung transplantation is the shortage of donor lungs. Only about 20% of donor lungs are accepted for transplantation. An *ex vivo* technique to evaluate and recondition lungs has been tested on donor lungs that have been rejected for transplantation with excellent results. This has resulted in an expansion of the donor pool with grafts from marginal donor lungs. Consequently, EVLP is being established as the new keystone in lung transplantation.

Since 2000, the first lung transplant *ex vivo* was performed by Steen *et al.* at our centre in Lund, Sweden, from a non-heart beating donor[12]. A few years later, a series of successful lung transplants were performed at our centre in Lund, Sweden by using grafts that did not meet standard transplantation criteria[152]. In the present study, we report our 10-year follow-up results regarding the short- and long-term outcome of the first six patients who underwent lung transplant using marginal donor lungs evaluated using EVLP at our centre. In addition, we compared this technique with conventional lung transplantation performed at our clinic during the same time period. Our EVLP protocols have been described in detail in our previous publications[152, 176].

We have previously reported our short-term EVLP experience with a 100% survival rate at 30 days, without significant differences shown between EVLP and standard lung transplantation regarding time on mechanical ventilation, ICU stay, or overall hospital stay[152, 154]. *Fildes et al.* reported up to 12 months' follow-up also without difference in mortality or incidence of infections between EVLP-lung transplantation and the conventional technique[185]. *Wallinder et al*., having one of the longest follow-ups with their 4-year experience, demonstrated that there is no superiority of conventional transplanted lungs over EVLP-lung transplant in terms of survival and postoperative complications[190]. Our 10-year experience is considered to be the longest clinical EVLP follow-up, which shows no significant difference between EVLP-transplanted lungs versus conventional transplant.

In the present study no significant difference was found in freedom from CLAD between EVLP-lung transplantation and the conventional technique. This finding is

in accordance with previous studies showing similar outcomes between the two groups concerning freedom from CLAD up to 5 years after lung trans-plantation[186, 190]. Additionally, this is the first time that the long-term outcome regarding pulmonary function has been investigated which showed no superiority between the two groups.

FEV_1 and 6MWT are well-known clinical tools that are non-invasive and provide excellent data on the clinical status of the recipient after transplantation[221].

EVLP has been suggested as a platform for administering medical agents and thus improving patient outcome[222, 223]. EVLP may also play a role in DCD, especially in uDCD donors in whom lung function is often unknown.

Interestingly it has been reported that patients who received DCD lungs that underwent EVLP showed an improvement in outcome regarding length of hospital stay and time on mechanical ventilation[189]. Extra preservation time could also reduce geographical limitations for recipients and donors, in addition to opening up possibilities for more daytime surgery[173, 224].

22.3 Paper III

The effect of graft ischaemic time on early graft function and long-term survival of patients who underwent lung transplantation remains under debate despite the fact that this topic has been studied widely. However, generally the consensus of the current studies is that the longer the ischaemic time, the greater the risk of severe reperfusion injury and PGD[44, 225, 226].

As described in the early and even in the current literature, the negative effect of cold ischaemic storage is mainly associated with a complicated pathogenesis process leading to cell death and graft injury[227].

Despite the relationship between ischaemic time and survival not being fully understood, clinicians usually attempt to avoid long geographical distances between the potential donor and recipients to reduce the risk of the negative effect of a long ischaemic time[227].

In the clinical setting, the expected time that the donor lungs may tolerate is related to multiple essential factors involving the donor organ (for example age, graft injury, cause of donor death). Generally, in lung transplantation, an ischaemic time of up to 6–8 hours is relatively acceptable, with an increased mortality in the first 30 days if the ischaemic time exceeds more than 8 hours[226, 227]. It has been reported that in

both paediatric and adult lung transplantation, a prolonged ischaemic time exceeding 6 hours in lung allografts does not limit survival, as was believed previously, with additional reports showing excellent results of lung transplantation for an ischaemic time of up to 12 hours[226].

As shown in our cohort, median ischaemic time in lung transplantation has increased steadily over the last 25 years, from less than 4 hours during the early 1990s to more than 5–6 hours currently. This is shown by a reduction of single lung transplants with an ischaemic time of 2–4 hours and an increase in double lung transplants with more than 6 hours of ischaemic time. The tendency towards double lung transplants in recent years is mainly explained by the overall superior survival and pulmonary function capacity shown in double lung transplants compared to single ones. Single lung transplant recipients comprise mainly COPD patients who typically are of greater age and have additional comorbidities, such as heart and vessel disease[43].

However, it must be recognised that an ischaemic time of around 2 hours is rare nowadays, and is usually the result of the donor organ being ready in the close geographical proximity, with a short and uncomplicated operative procedure often involving single lung transplant.

Interestingly it was shown that in the case of a lower ischaemic time of between 2 and 4 hours, the patient has a superior survival when compared to more than 4 hours of ischaemic time that is usually the case for double lung transplantation. Furthermore, we were able to demonstrate in our study that a 2-hour difference in ischaemic time for patients with a limited survival of up to 1 year increases the mortality by as much as up to 25%.

Our cohort has also shown uniquely that there is an increase in the cumulative incidence of death of almost 15%, and even higher in emphysema patients with almost double the incidence.

Thabut et al. reported the harmful effect of ischaemic time peaking in the first year, which then diminishes in long-term follow-up[228].

As the present study has been able to show, regarding the hazard from each 2-hour window of ischaemic time, it may be hypothesised that using EVLP as early as possible could improve the outcome after lung transplantation by minimising the hazard from a prolonged ischaemic time and thus result in better graft survival.

With the rise of mobile EVLP devices, such as the OCS, the harmful effects of ischaemic time that limit postoperative survival after lung transplantation could be prevented. These parameters were, however, not studied in this study, but may be relevant for future analyses.

22.4 Paper IV

PEA analysed at the time of making the underlying diagnosis is associated with a trend of grouping of patients with CF and alpha-1 antitrypsin deficiency, as well as BOS grades grouped together, especially grades 0 and 1. This demonstrates the potential for further biomarkers for these diseases. The CF grouping consisted of various BOS grades; further work could utilise this pattern of detected proteins to predict progression in rejection severity post-transplantation.

This study demonstrated a drop in CRH levels as the grades of BOS increased. These changes were not found to correlate with immunosuppression therapy or patient characteristics. The drop in CRH levels across all BOS grades, found by using PEA, was validated by ELISA at baseline and the 1-year follow up with similar results. In support of its potential as a biomarker, CRH levels were unchanged in patients who maintained a BOS grade of 0 between their baseline and follow-up after 1 year; however, a significant drop in plasma concentration of CRH was observed in the progress of the BOS grade at follow-up. This supports the hypothesis that CRH measurements have the potential to reflect an increasing risk of BOS.

CRH is known as a major integrator of endocrine, autonomic, and immune responses to stress[229-231]. Its most prominent role is as the hypothalamic regulator of adrenocorticotropic hormone (ACTH) secretion which stimulates adrenal cortisol synthesis as an anti-inflammatory hormone[232].

Locally produced CRH in peripheral tissues, including the lungs[233, 234], indicates a direct role in facilitating the inflammatory responses. The distal actions of CRH via cortisol are anti-inflammatory, while the direct action of CRH in peripheral tissues is pro-inflammatory.

CRH has also been shown to stimulate mast cell degranulation, T-lymphocyte proliferation, antibody production, natural killer cell activity, leukocyte chemotaxis, vascular permeability, and the expression of cytokines and ROS metabolites[230]. CRH has been linked to lung mechanical dysfunction, and a lack of CRH has been associated with an increase in allergen-induced airway inflammation in asthma[235]. This suggests that CRH can function in the management of immune and inflammatory responses.

Other mediators of inflammation have been singled out as potential biomarkers, including IL-1RA, but CRH is a novel candidate.

In order to confirm the relationship of the decrease of CRH in BOS patients seen in this study, we used gene expression data from transbronchial biopsies of lung tissue. In a microarray of 457 biopsies, there was a difference between the higher non-CLAD CRH values and the lower CLAD values (22 versus 21, p=0.042). This reinforces the conclusion drawn here that CRH has potential as a biomarker for chronic graft rejection.

Limitations of this study include the relatively small sample size, as well as the limited follow-up. Given the course of BOS and rates of survival following transplantation, a 1-year follow-up was initiated as a starting point to begin to uncover potential differences and biomarkers that could occur in diseased and non-diseased patients. Further work could include a longer period in which to track the cohorts to determine relative changes in the proteins as patient health conditions were either maintained or showed a deterioration. In this study, microarray data collected from samples across 10 centres supported the findings of lower CRH levels in the BOS patient group. The use of PEA to find the relative plasma concentrations of CRH in other patient cohorts at more centres would help support the findings of this current study.

22.5 Paper IV

The results suggest that MSC treatment improves the gas exchange capacity during EVLP. Additionally, the treatment showed an ameliorating effect in pulmonary function during the 72 hours' follow-up, and the capacity to decrease the incidence of PGD in the recipient. Previous studies have demonstrated that MSCs may ameliorate pulmonary function in ALI and ARDS[236-238]. However, this is the first study to investigate the potential of MSC therapy in restoring aspiration-injured lungs with the aim of increasing the donor pool and decreasing the incidence of PGD.

Critical for this study was the establishment of ALI. The gastric content-induced lung injury model represents an opportunity to explore the expansion of the donor pool, considering the large number of lungs rejected due to this type of injury[61].

Pneumonitis or pneumonia due to aspiration is a common cause for ALI and ARDS in the ICU, as well as in donor lungs[239, 240].

This study employed a lung injury model in which gastric content was administered endotracheally to induce ARDS. Aspiration induces a chemical insult to the lung parenchyma and the airways. Following the injury, a cascade of inflammatory

responses takes place, during which various inflammatory mediators are released, resulting in diffuse alveolar damage and progressive hypoxaemia[241].

The lung injury can be characterised by a biphasic response. The first phase comprises an early insult and direct caustic actions of the low pH on the lung epithelium; the second phase by an acute neutrophilic inflammatory response at 4–6 hours. Key mediators in the inflammatory response are TNFα and IL-8[240].

In the present study, gastric content was administered equally throughout the lung lobes using bronchoscopy. Six hours after the first dose was given, all pigs had developed infiltration as seen on thoracic imaging and by decreased gas exchange capacity. This lung injury was then confirmed retrospectively with histo-pathological analysis. Furthermore, there were no significant differences in the severity of the lung injury between treated and non-treated animals after lung injury had been established.

The damage model can be emphasised by the significantly elevated PVR following gastric content administration, which is associated with lung injury and known as a negative prognostic factor[242].

EVLP provides a treatment platform opportunity. In the present study, human bone marrow-derived MSCs were given during EVLP.

All lungs still had signs of ALI with poor oxygenation when connected to the EVLP. One dose of MSCs was given in this study during EVLP, which was associated with a significant increase in the oxygenation capacity by the end of the 4 hours in EVLP when compared to the degree of oxygenation at the initiation of EVLP. In contrast, there were no significant changes observed from the start to the conclusion of EVLP within the non-treated group.

Importantly, however, in order for a donor graft to be considered acceptable for transplantation, the graft must reach a minimum of 300 mmHg in the PaO_2/FiO_2 ratio[243], a criterion which was met for four of the six treated grafts. This criterion was not met for any of the non-treated donor grafts.

Other work has been carried out to establish how the addition of MSCs to the EVLP circuit can improve the damaged lungs, which has demonstrated that the MSC treatment can increase the alveolar fluid clearance rate and reduce oedema[103]. After EVLP, two further doses of MSCs were administered post-lung transplant. This was followed by post-lung transplant monitoring, which concluded that a right pulmectomy of the native lung enables a means of exclusively evaluating the left transplanted lung.

Lung function and PGD were evaluated in this period, which was 72 hours' post-lung transplant and the treated group showed considerable improvement at this point compared to the non-treated group. The treated group showed an improved gas exchange capacity, which was significantly better when compared to the recipients in the non-treated group. The degree of PGD among the recipients was further determined at 72 hours' post-lung transplant. None of the treated group had developed PGD at this point, which led to an assigned PGD grade of 0. In contrast, five of the six non-treated recipients had PGD grades 2–3. This demonstrates that the MSC treatment was able to decrease postoperative graft dysfunction.

In this study, live cell treatment with human bone marrow-derived MSCs was well tolerated with no observed adverse effects. The isolation and expansion of the MSC treatment in this study were not subjected to GMP facility conditions; however, the cells did undergo quality control along with characterisation of cell marker expression. GMP conditions could be implemented as the work transitioned from the translational study phase to the clinic. Other limitations of this study include a low sample size; however, the study was powered adequately to determine statistical significance within the measured parameters. Anatomical challenges in the porcine right bronchus prohibit transplantation of the right lung, limiting the model to single lung transplantation[244]. This was addressed through the use of a right pneumonectomy at the conclusion of the experimental monitoring period to allow for the study of the left transplanted lung alone.

23 Ethical aspects

Paper I
The study was approved by the local Ethics Committee for Animal Research (Dnr 5.2.18-4903/16, and Dnr 5.2.18-8927/16) at Lund University. All animals received care according to the USA Principles of Laboratory Animal Care of the National Society for Medical Research, Guide for the Care and Use of Laboratory Animals, National Academies Press (1996). All animal handling, welfare monitoring, and euthanasia were attended to according to the guide for laboratory animals under the supervision of an on-site veterinarian.

Papers II, III
This study was performed in accordance with the Declaration of Helsinki and is approved by the Ethics Committee at Lund University with reference number 2016/638. All patients gave their written consent to participate.

Paper IV
The study was performed in accordance with the Declaration of Helsinki and was approved by the Swedish Ethical Board (Dnr 2017/396). All patients gave written, informed consent before entering the study.

Paper V
Approval for conducting this study was given by the local Ethics Committee (Dnr 8401/2017). All animals were given care according to the USA principles of Laboratory Animal Care of the National Society for Medical Research, Guide for the Care and Use of Laboratory Animals, National Academies Press (1996).

24 Conclusions

24.1 Paper I

This study showed that the utilisation of the cytokine adsorption filter led to the acceptance of more lungs for transplantation and also increased the tolerability of lungs in a recipient through: (1) reduction of inflammation and restoration of pulmonary function during EVLP, (2) rejuvenation of function and decrease in inflammation following transplantation, and (3) reduction in the incidence of PGD in transplanted recipients.

24.2 Paper II

On average 40% of BDD lungs do not meet the criteria for lung transplantation and are therefore not accepted. A considerable number of these organs may have been utilised in lung transplantation through EVLP, which provides a method of evaluating and improving marginal donor lungs. According to the findings of our 10-year follow-up — the longest clinical follow-up to date — no differences were found between conventional lung transplantation and EVLP regarding survival, pulmonary function, or incidence of CLAD.

24.3 Paper III

The acceptable limits of the ischaemic time within lung transplantation are still under discussion in the current literature, with a consensus that the longer the ischaemic time, the greater the complications. Despite this, in lung transplantation an ischaemic time of up to 8 hours is deemed acceptable, despite conflicting data. Our study showed that every 2-hour increase of ischaemic time proves equivalent to an increased mortality of up to 24% within 5 years. Recipients with an ischaemic time of 2 hours had a better survival (1 and 5 years) after lung transplantation

compared to patients with an ischaemic time of up to 6 hours. In the 25-year follow-up, no difference was shown in recipients for whom ischaemic time ranged between 2 and 6 hours or more; however, patients with an ischaemic time of 2 hours were still able to maintain their superiority compared to other ischaemic time groups in the 25-year follow-up, despite several other disadvantages.

24.4 Paper IV

There is convincing evidence in the literature outlining the role of CRH in the modulation of inflammation as well as its link to lung dysfunction. In the current study, decreases in CRH levels were observed in patients who developed BOS. These CRH deficiencies were not only shown in patients with BOS grade 1 but also in patients with the more severe grades 2 and 3. This reflects the importance of a CRH depletion across early and late processes of BOS development and helps to identify a potential marker as a novel diagnostic tool.

24.5 Paper V

In summary, this current study demonstrates the potential of using MSC treatment in the context of EVLP and postoperatively to restore aspiration-injured donor lungs, rendering them suitable for transplantation. In the current study, pulmonary graft function improved significantly in the treated lungs both after EVLP and after lung transplantation. Furthermore, the MSC treatment significantly decreased the incidence of PGD in the recipients. This shows the potential of MSCs as a promising therapeutic option for regenerating severely damaged donor lungs. The results imply that the treatment could be used to both increase the donor pool and decrease postoperative complications.

25 Future perspectives

As described in this thesis, lung transplantation is the only treatment option for severe terminal lung disease. Receiving new lungs means getting a new life, but unfortunately in many people tissue rejection of the new lungs occurs and after 5 years, only about half of the patients are still alive. The rejection starts as soon as the organ is in the recipient. Despite immunosuppressive medication, up to 70% of the new lungs have difficulty in being tolerated by the body, and to enable the body to accept the new lung is therefore of great importance.

The early intolerance to the new lung begins at the time of transplantation and is driven by an immuno-inflammatory process in the body, similar to the inflammatory process that occurs in infectious diseases, such as pneumonia and sepsis. For septic conditions, it is now clinical practice to use cytokine adsorption to reduce the strong inflammation in the body. This has also been shown to be very effective in Covid-19 infections. In heart transplantation, a cytokine adsorption filter has also been used in a clinical study and results showed that the body's toleration of the new heart was improved.

In our preclinical study, we have shown that cytokine infiltration significantly increases the body's tolerance for the new lung and reduces post-transplantation PGD. These results formed the basis for our idea of our current clinical study, which is a randomised controlled trial in which lung transplant patients are randomised to receive cytokine adsorption or not during the first day after lung transplantation. Our study's aim is to increase the patient's tolerance for the new lung during the transplant and thus reduce PGD and CLAD later, which means increasing the patient's chance of a long life with good lung function after their lung transplant.

In the future, it would also be interesting to extend our aspiration-induced ALI models under the hypothesis that MSC therapy could improve aspiration-damaged lungs and reduce the incidence of PGD. We would add a new treated group which would comprise lungs with aspiration-induced ARDS receiving EVLP without MSC treatment, but MSC treatment would only be given post-transplantation. We would like to investigate this and compare results between the groups (one-step group, two-step group in addition to the control group).

26 Acknowledgements

I would like to express my sincere appreciation to my fellow authors for the studies upon which this thesis is based. This thesis has been accomplished thanks to the help and support of countless people who have supported and encouraged me along the way, to whom I owe my gratitude.

To all patients who have participated in the studies. I am also respectfully grateful towards the animals that have sacrificed their lives in order for us to carry out this important research.

Professor ***Sandra Lindstedt***, main supervisor, my dear colleague, and friend. I will really forever be grateful that you invited me to become a part of your research team and for believing in me from the very beginning, although my prior research experience was simple and basic. You have introduced me to the world of research in the best possible way. I have been extremely lucky to have a supervisor who is an icon of integrity and hard work. Thanks once again for all your encouragement and support.

I would like also to thank my co-supervisors, ***Leif Pierre*** and ***Snejana Hyllén***, for challenging me and supporting me. Their input and patience has been invaluable in helping me to learn how to conduct research, and to navigate my way through some of the more emotionally challenging aspects of this project.

I would like to thank Professor ***Richard Ingemansson*** for his helpful practical advice and unceasing ideas that have helped me at all times in my research career.

I am extremely grateful to my best friend and colleague ***Mohammed Fakhro***, who was always there when I needed help and always managed to inspire me with his knowledge and motivation.

From the bottom of my heart, I would like to say a big thank you for the amazing preclinical research group members for their energy, understanding and help throughout my project.

I would like to extend my sincere thanks to ***Franziska Olm and Anna Niroomand*** without your help, this project would have not been the same!

A special thanks to my co-authors ***Martin Stenlo, Dag Edström, Margareta Mittendorfer, Gabriel Hirdman, Ellen Broberg, Jesper Andreasson, Oskar Hallgren*** and all preclinical research group in BMC. You are all aware of who you are and your invaluable contributions and that words are not enough to express my sincere gratitude for your support.

I also would like to say a special thank you to ***Evamarie Braf*** for your hard work during laboratory weeks, thanks once again for all your perfect coordination and arrangement.

I would like to express my deepest appreciation to my colleagues in our general thoracic surgery section and especially grateful to ***Dr. Alaa Abdulahad,*** our scheduler (schemaläggare), for supporting me. A special thanks to our section's secretary ***My Linde Persson.*** I am so grateful for your help.

I would also like to thank all the co-authors and all co-workers at the Department of Cardiothoracic Surgery, Pulmonary Medicine and Thoracic Intensive Care and Anaesthesia, Skåne University Hospital, Lund University, Sweden.

I would like to express my special appreciation and thanks to Assistant Professor ***Bengt Åberg.*** I am so grateful that you took me under your wing when I first started working in Sweden. Your leadership has helped me fulfil my potential. I would not be where I am today without you.

And last, but not least , my biggest thanks go out to my wife ***Saba,*** thanks for all your support, without your help I would have terminated these studies a long time ago. Also, I would like to thank my children, ***Hasanin, Rosa and Maya,*** without their tremendous understanding and encouragement in the past few years, it would have been impossible for me to complete my thesis.

(From left) Supervisor Professor Sandra Lindstedt, Co-supervisor: Snejana Hyllén, Co-supervisor: Leif Pierre and Haider Ghaidan

Photo:Ludvig Thunman /Bildbyrån www.dagensmedicine.se

27 References

1 Ochs M, Nyengaard JR, Jung A et al. The number of alveoli in the human lung. American journal of respiratory and critical care medicine 2004; 169 (1): 120-124.

2 Duffin J. Role of acid-base balance in the chemoreflex control of breathing. Journal of applied physiology 2005; 99 (6): 2255-2265.

3 Townsley MI. Structure and composition of pulmonary arteries, capillaries and veins. Comprehensive Physiology 2012; 2: 675.

4 Whitener S, Konoske R, Mark JB. Pulmonary artery catheter. Best Practice & Research Clinical Anaesthesiology 2014; 28 (4): 323-335.

5 Swan H, Ganz W, Forrester J et al. Catheterization of the heart in man with use of a flow-directed balloon-tipped catheter. New England Journal of Medicine 1970; 283 (9): 447-451.

6 Van Trigt P, Davis RD, Shaeffer GS et al. Survival benefits of heart and lung transplantation. Annals of surgery 1996; 223 (5): 576.

7 Hardy JD, Webb WR, Dalton ML et al. Lung homotransplantation in man: report of the initial case. Jama 1963; 186 (12): 1065-1074.

8 Magovern GJ, Yates AJ. Human homotransplantation of left lung: report of a case. Annals of the New York Academy of Sciences 1965; 120 (2): 710-728.

9 Derom F, Barbier F, Ringoir S et al. Ten-month survival after lung homotransplantation in man. The Journal of thoracic and cardiovascular surgery 1971; 61 (6): 835-846.

10 Reitz BA, Wallwork JL, Hunt SA et al. Heart-lung transplantation: successful therapy for patients with pulmonary vascular disease. New England Journal of Medicine 1982; 306 (10): 557-564.

11 Patterson G, Cooper J, Goldman B et al. Technique of successful clinical double-lung transplantation. The Annals of thoracic surgery 1988; 45 (6): 626-633.

12 Steen S, Sjöberg T, Pierre L et al. Transplantation of lungs from a non-heart-beating donor. The Lancet 2001; 357 (9259): 825-829.

13 Thomas M, Martin AK, Allen WL et al. Lung transplantation using a hybrid extracorporeal membrane oxygenation circuit. ASAIO Journal 2020; 66 (10): e123-e125.

14 Aigner C, Wisser W, Taghavi S et al. Institutional experience with extracorporeal membrane oxygenation in lung transplantation. European journal of cardio-thoracic surgery 2007; 31 (3): 468-474.

15 McGiffin DC, Alonso JE, Zorn Jr GL et al. Sternal approximation for bilateral anterolateral transsternal thoracotomy for lung transplantation. The Annals of Thoracic Surgery 2005; 79 (2): e19-e20.

16 Boasquevisque CHR, Yildirim E, Waddel TK et al. Surgical techniques: lung transplant and lung volume reduction. Proceedings of the American Thoracic Society 2009; 6 (1): 66-78.

17 Hayanga JA, D'Cunha J. The surgical technique of bilateral sequential lung transplantation. Journal of thoracic disease 2014; 6 (8): 1063.

18 Mody GN, Coppolino A, Singh SK et al. Sternotomy versus thoracotomy lung transplantation: key tips and contemporary results. Annals of cardiothoracic surgery 2020; 9 (1): 60.

19 Calhoon JH, Grover FL, Gibbons WJ et al. Single lung transplantation: alternative indications and technique. The Journal of thoracic and cardiovascular surgery 1991; 101 (5): 816-825.

20 Antończyk R, Stącel T, Urlik M et al. Single lung transplant vs double lung transplant: a single-center experience with particular consideration for idiopathic pulmonary arterial hypertension. *Transplantation Proceedings.* 52. Elsevier; 2020:2138-2142.

21 Elgharably H, Javorski MJ, McCurry KR. Bilateral sequential lung transplantation: technical aspects. Journal of Thoracic Disease 2021; 13 (11): 6564.

22 Date H. Living-related lung transplantation. Journal of Thoracic Disease 2017; 9 (9): 3362.

23 Shah RJ, Diamond JM. Primary graft dysfunction (PGD) following lung transplantation. *Seminars in respiratory and critical care medicine.* 39. Thieme Medical Publishers; 2018:148-154.

24 Kuntz CL, Hadjiliadis D, Ahya VN et al. Risk factors for early primary graft dysfunction after lung transplantation: a registry study. Clinical transplantation 2009; 23 (6): 819-830.

25 Liu Y, Liu Y, Su L et al. Recipient-related clinical risk factors for primary graft dysfunction after lung transplantation: a systematic review and meta-analysis. PLoS One 2014; 9 (3): e92773.

26 Lee JC, Christie JD. Primary graft dysfunction. Proceedings of the American Thoracic Society 2009; 6 (1): 39-46.

27 Christie JD, Bavaria JE, Palevsky HI et al. Primary graft failure following lung transplantation. Chest 1998; 114 (1): 51-60.

28 Meyers BF, De La Morena M, Sweet SC et al. Primary graft dysfunction and other selected complications of lung transplantation: a single-center experience of 983 patients. The Journal of thoracic and cardiovascular surgery 2005; 129 (6): 1421-1429.

29 Kshettry VR, Kroshus TJ, Hertz MI et al. Early and late airway complications after lung transplantation: incidence and management. The Annals of thoracic surgery 1997; 63 (6): 1576-1583.

30 Susanto I, Peters JI, Levine SM et al. Use of balloon-expandable metallic stents in the management of bronchial stenosis and bronchomalacia after lung transplantation. Chest 1998; 114 (5): 1330-1335.

31 Li Ng Y, Paul N, Patsios D et al. Imaging of lung transplantation. American Journal of Roentgenology 2009; 192 (3_supplement): S1-S13.

32 Verleden SE, Vasilescu DM, Willems S et al. The site and nature of airway obstruction after lung transplantation. American journal of respiratory and critical care medicine 2014; 189 (3): 292-300.

33 Kramer MR, Marshall SE, Starnes VA et al. Infectious complications in heart-lung transplantation: analysis of 200 episodes. Archives of internal medicine 1993; 153 (17): 2010-2016.

34 Gauthier JM, Hachem RR, Kreisel D. Update on chronic lung allograft dysfunction. Current transplantation reports 2016; 3 (3): 185-191.

35 Christie JD, Carby M, Bag R et al. Report of the ISHLT working group on primary lung graft dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. The Journal of heart and lung transplantation 2005; 24 (10): 1454-1459.

36 Royer P-J, Olivera-Botello G, Koutsokera A et al. Chronic lung allograft dysfunction: a systematic review of mechanisms. Transplantation 2016; 100 (9): 1803-1814.

37 Vandermeulen E, Lammertyn E, Verleden SE et al. Immunological diversity in phenotypes of chronic lung allograft dysfunction: a comprehensive immunohistochemical analysis. Transplant International 2017; 30 (2): 134-143.

38 Health UDo, Services H. 1998 Annual Report of the US Scientific Registry for Transplant Recipients and the Organ Procurement and Transplantation Network: Transplant data 1988-1998. 1999.

39 Thabut G, Mal H. Outcomes after lung transplantation. Journal of thoracic disease 2017; 9 (8): 2684.

40 Leard LE, Holm AM, Valapour M et al. Consensus document for the selection of lung transplant candidates: An update from the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2021; 40 (11): 1349-1379.

41 Maurer JR, Frost AE, Estenne M et al. International guidelines for the selection of lung transplant candidates. Transplantation 1998; 66 (7): 951-956.

42 Orens JB, Estenne M, Arcasoy S et al. International guidelines for the selection of lung transplant candidates: 2006 update—a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. The Journal of heart and lung transplantation 2006; 25 (7): 745-755.

43 Fakhro M, Ingemansson R, Skog I et al. 25-year follow-up after lung transplantation at Lund University Hospital in Sweden: superior results obtained for patients with cystic fibrosis. Interactive cardiovascular and thoracic surgery 2016; 23 (1): 65-73.

44 Chambers DC, Perch M, Zuckermann A et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-eighth adult lung transplantation report—2021; focus on recipient characteristics. The Journal of Heart and Lung Transplantation 2021; 40 (10): 1060-1072.

45 Leard LE, Holm AM, Valapour M et al. Consensus document for the selection of lung transplant candidates: An update from the International Society for Heart and Lung Transplantation. The Journal of Heart and Lung Transplantation 2021; 40 (11): 1349-1379.

46 Kapnadak SG, Raghu G. Lung transplantation for interstitial lung disease. European Respiratory Review 2021; 30 (161).

47 Lehr CJ, Blackstone EH, McCurry KR et al. Extremes of age decrease survival in adults after lung transplant. Chest 2020; 157 (4): 907-915.

48 Shigemura N, Toyoda Y. Elderly patients with multiple comorbidities: insights from the bedside to the bench and programmatic directions for this new challenge in lung transplantation. Transplant International 2020; 33 (4): 347-355.

49 Yusen RD, Edwards LB, Kucheryavaya AY et al. The registry of the International Society for Heart and Lung Transplantation: thirty-first adult lung and heart–lung transplant report—2014; focus theme: retransplantation. The Journal of Heart and Lung Transplantation 2014; 33 (10): 1009-1024.

50 Renard R, Girault A, Avramenko-Bouvier A et al. Outcome of lung transplantation using grafts from donors over 65 years of age. The Annals of Thoracic Surgery 2021; 112 (4): 1142-1149.

51 Chambers DC, Cherikh WS, Goldfarb SB et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-fifth adult lung and heart-lung transplant report—2018; focus theme: multiorgan transplantation. The Journal of Heart and Lung Transplantation 2018; 37 (10): 1169-1183.

52 Weill D, Benden C, Corris PA et al. A consensus document for the selection of lung transplant candidates: 2014—an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. The Journal of Heart and Lung Transplantation 2015; 34 (1): 1-15.

53 van der Mark SC, Hoek RA, Hellemons ME. Developments in lung transplantation over the past decade. European Respiratory Review 2020; 29 (157).

54 Khandhar SJ, Althouse AD, Mulukutla S et al. Postoperative outcomes and management strategies for coronary artery disease in patients in need of a lung transplantation. Clin Transplant 2017; 31 (9).

55 Chaikriangkrai K, Jyothula S, Jhun HY et al. Impact of pre-operative coronary artery disease on cardiovascular events following lung transplantation. J Heart Lung Transplant 2016; 35 (1): 115-121.

56 Young KA, Dilling DF. The future of lung transplantation. Chest 2019; 155 (3): 465-473.

57 Sage E, Mussot S, Trebbia G et al. Lung transplantation from initially rejected donors after ex vivo lung reconditioning: the French experience. European Journal of Cardio-Thoracic Surgery 2014; 46 (5): 794-799.

58 Lo-Cao E, Hall S, Parsell R et al. Neurogenic pulmonary edema. The American Journal of Emergency Medicine 2021; 45: 678. e673-678. e675.

59 Marik PE. Aspiration pneumonitis and aspiration pneumonia. New England Journal of Medicine 2001; 344 (9): 665-671.

60 Sundaresan S, Semenkovich J, Ochoa L et al. Successful outcome of lung transplantation is not compromised by the use of marginal donor lungs. The Journal of thoracic and cardiovascular surgery 1995; 109 (6): 1075-1080.

61 Meers C, Van Raemdonck D, Verleden GM et al. The number of lung transplants can be safely doubled using extended criteria donors; a single-center review. Transplant International 2010; 23 (6): 628-635.

62 Orens JB, Boehler A, De Perrot M et al. A review of lung transplant donor acceptability criteria. The Journal of heart and lung transplantation 2003; 22 (11): 1183-1200.

63 Botha P. Extended donor criteria in lung transplantation. Current opinion in organ transplantation 2009; 14 (2): 206-210.

64 Aigner C, Winkler G, Jaksch P et al. Extended donor criteria for lung transplantation—a clinical reality. European journal of cardio-thoracic surgery 2005; 27 (5): 757-761.

65 Hoang V, Medrek SK, Pendurthi M et al. Improving donor lung management and recipient selection in lung transplantation. American Journal of Respiratory and Critical Care Medicine 2017; 196 (6): 782-784.

66 Woolley AE, Singh SK, Goldberg HJ et al. Heart and lung transplants from HCV-infected donors to uninfected recipients. New England Journal of Medicine 2019; 380 (17): 1606-1617.

67 Machuca TN, Cypel M. Ex vivo lung perfusion. Journal of thoracic disease 2014; 6 (8): 1054.

68 Possoz J, Neyrinck A, Van Raemdonck D. Ex vivo lung perfusion prior to transplantation: an overview of current clinical practice worldwide. Journal of Thoracic Disease 2019; 11 (4): 1635.

69 Valenza F, Rosso L, Coppola S et al. Ex vivo lung perfusion to improve donor lung function and increase the number of organs available for transplantation. Transplant International 2014; 27 (6): 553-561.

70 Chan PG, Kumar A, Subramaniam K et al. Ex Vivo Lung Perfusion: A Review of Research and Clinical Practices. Semin Cardiothorac Vasc Anesth 2020; 24 (1): 34-44.

71 Nakajima D, Nagata S, Kayawake H et al. Successful lung transplant cases with ex vivo lung perfusion assessment of extended criteria donor lungs. Gen Thorac Cardiovasc Surg 2022; 70 (4): 406-412.

72 Ehrsam JP, Benden C, Immer FF et al. Current status and further potential of lung donation after circulatory death. Clinical Transplantation 2021; 35 (7): e14335.

73 Thuong M, Ruiz A, Evrard P et al. New classification of donation after circulatory death donors definitions and terminology. Transplant International 2016; 29 (7): 749-759.

74 Assadiasl S, Nicknam MH. Cytokines in Lung Transplantation. Lung 2022; 200 (6): 793-806.

75 Verleden GM, Glanville AR, Lease ED et al. Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment—A consensus report from the Pulmonary Council of the ISHLT. The Journal of Heart and Lung Transplantation 2019; 38 (5): 493-503.

76 Sato M. Chronic lung allograft dysfunction after lung transplantation: the moving target. General thoracic and cardiovascular surgery 2013; 61 (2): 67-78.

77 Berastegui C, Gomez-Olles S, Sanchez-Vidaurre S et al. BALF cytokines in different phenotypes of chronic lung allograft dysfunction in lung transplant patients. Clin Transplant 2017; 31 (3).

78 Vanaudenaerde BM, De Vleeschauwer SI, Vos R et al. The role of the IL23/IL17 axis in bronchiolitis obliterans syndrome after lung transplantation. Am J Transplant 2008; 8 (9): 1911-1920.

79 Wen XH, Kong HY, Zhu SM et al. Plasma levels of tumor necrotic factor-alpha and interleukin-6,-8 during orthotopic liver transplantation and their relations to postoperative pulmonary complications. Hepatobiliary & pancreatic diseases international: HBPD INT 2004; 3 (1): 38-41.

80 Pham S, Yoshida Y, Aeba R et al. Interleukin-6, a marker of preservation injury in clinical lung transplantation. The Journal of heart and lung transplantation: the official publication of the International Society for Heart Transplantation 1992; 11 (6): 1017-1024.

81 Moreno I, Vicente R, Ramos F et al. Determination of interleukin-6 in lung transplantation: association with primary graft dysfunction. *Transplantation proceedings.* 39. Elsevier; 2007:2425-2426.

82 Mathur A, Baz M, Staples ED et al. Cytokine profile after lung transplantation: correlation with allograft injury. The Annals of thoracic surgery 2006; 81 (5): 1844-1850.

83 SCHOLMA J, SLEBOS D-J, Marike boezen H et al. Eosinophilic granulocytes and interleukin-6 level in bronchoalveolar lavage fluid are associated with the development of obliterative bronchiolitis after lung transplantation. American journal of respiratory and critical care medicine 2000; 162 (6): 2221-2225.

84 de Perrot M, Young K, Imai Y et al. Recipient T cells mediate reperfusion injury after lung transplantation in the rat. The Journal of Immunology 2003; 171 (10): 4995-5002.

85 FISHER AJ, DONNELLY SC, HIRANI N et al. Elevated levels of interleukin-8 in donor lungs is associated with early graft failure after lung transplantation. American journal of respiratory and critical care medicine 2001; 163 (1): 259-265.

86 De Perrot M, Sekine Y, Fischer S et al. Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. American journal of respiratory and critical care medicine 2002; 165 (2): 211-215.

87 Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. Immunological reviews 2008; 226 (1): 205-218.

88 de Perrot M, Fischer S, Liu M et al. Impact of human interleukin-10 on vector-induced inflammation and early graft function in rat lung transplantation. American journal of respiratory cell and molecular biology 2003; 28 (5): 616-625.

89 Eppinger MJ, Ward PA, Bolling SF et al. Regulatory effects of interleukin-10 on lung ischemia-reperfusion injury. The Journal of thoracic and cardiovascular surgery 1996; 112 (5): 1301-1306.

90 Welborn MB, Oldenburg HS, Hess PJ et al. The relationship between visceral ischemia, proinflammatory cytokines, and organ injury in patients undergoing thoracoabdominal aortic aneurysm repair. Critical Care Medicine 2000; 28 (9): 3191-3197.

91 Hawchar F, Laszlo I, Öveges N et al. Extracorporeal cytokine adsorption in septic shock: a proof of concept randomized, controlled pilot study. Journal of critical care 2019; 49: 172-178.

92 Schädler D, Pausch C, Heise D et al. The effect of a novel extracorporeal cytokine hemoadsorption device on IL-6 elimination in septic patients: a randomized controlled trial. PloS one 2017; 12 (10): e0187015.

93 Rieder M, Wengenmayer T, Staudacher D et al. Cytokine adsorption in patients with severe COVID-19 pneumonia requiring extracorporeal membrane oxygenation. Critical Care 2020; 24 (1): 1-2.

94 Peng Z-Y, Carter MJ, Kellum JA. Effects of hemoadsorption on cytokine removal and short-term survival in septic rats. Critical care medicine 2008; 36 (5): 1573.

95 Peng Z-Y, Wang H-Z, Carter MJ et al. Acute removal of common sepsis mediators does not explain the effects of extracorporeal blood purification in experimental sepsis. Kidney international 2012; 81 (4): 363-369.

96 Kellum JA, Song M, Venkataraman R. Hemoadsorption removes tumor necrosis factor, interleukin-6, and interleukin-10, reduces nuclear factor-κB DNA binding, and improves short-term survival in lethal endotoxemia. Critical care medicine 2004; 32 (3): 801-805.

97 Ferdinand JR, Hosgood SA, Moore T et al. Cytokine absorption during human kidney perfusion reduces delayed graft function-associated inflammatory gene signature. Am J Transplant 2021; 21 (6): 2188-2199.

98 Nemeth E, Kovacs E, Racz K et al. Impact of intraoperative cytokine adsorption on outcome of patients undergoing orthotopic heart transplantation—an observational study. Clinical Transplantation 2018; 32 (4): e13211.

99 Kakishita T, Oto T, Hori S et al. Suppression of inflammatory cytokines during ex vivo lung perfusion with an adsorbent membrane. Ann Thorac Surg 2010; 89 (6): 1773-1779.

100 Iskender I, Cosgun T, Arni S et al. Cytokine filtration modulates pulmonary metabolism and edema formation during ex vivo lung perfusion. J Heart Lung Transplant 2017.

101 Iskender I, Arni S, Maeyashiki T et al. Perfusate adsorption during ex vivo lung perfusion improves early post-transplant lung function. The Journal of Thoracic and Cardiovascular Surgery 2021; 161 (2): e109-e121.

102 Friedenstein AJ, Chailakhyan RK, Latsinik NV et al. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 1974; 17 (4): 331-340.

103 Lee JW, Fang X, Gupta N et al. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci U S A 2009; 106 (38): 16357-16362.

104 Fang X, Abbott J, Cheng L et al. Human Mesenchymal Stem (Stromal) Cells Promote the Resolution of Acute Lung Injury in Part through Lipoxin A4. J Immunol 2015; 195 (3): 875-881.

105 Lalu MM, McIntyre L, Pugliese C et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One 2012; 7 (10): e47559.

106 Matthay MA, Calfee CS, Zhuo H et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. Lancet Respir Med 2019; 7 (2): 154-162.

107 Lee OK, Kuo TK, Chen W-M et al. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004; 103 (5): 1669-1675.

108 Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem cells 2003; 21 (1): 105-110.

109 Miao Z, Jin J, Chen L et al. Isolation of mesenchymal stem cells from human placenta: comparison with human bone marrow mesenchymal stem cells. Cell biology international 2006; 30 (9): 681-687.

110 Maqsood M, Kang M, Wu X et al. Adult mesenchymal stem cells and their exosomes: Sources, characteristics, and application in regenerative medicine. Life Sciences 2020; 256: 118002.

111 Mordant P, Nakajima D, Kalaf R et al. Mesenchymal stem cell treatment is associated with decreased perfusate concentration of interleukin-8 during ex vivo perfusion of donor lungs after 18-hour preservation. The Journal of Heart and Lung Transplantation 2016; 35 (10): 1245-1254.

112 Martens A, Ordies S, Vanaudenaerde BM et al. Immunoregulatory effects of multipotent adult progenitor cells in a porcine ex vivo lung perfusion model. Stem cell research & therapy 2017; 8 (1): 1-9.

113 Nakajima D, Watanabe Y, Ohsumi A et al. Mesenchymal stromal cell therapy during ex vivo lung perfusion ameliorates ischemia-reperfusion injury in lung transplantation. The Journal of Heart and Lung Transplantation 2019; 38 (11): 1214-1223.

114 Mowery NT, Terzian WH, Nelson AC. Acute lung injury. Current problems in surgery 2020; 57 (5): 100777.

115 Ware LB, Matthay MA. The acute respiratory distress syndrome. New England Journal of Medicine 2000; 342 (18): 1334-1349.

116 Milberg JA, Davis DR, Steinberg KP et al. Improved survival of patients with acute respiratory distress syndrome (ARDS): 1983-1993. Jama 1995; 273 (4): 306-309.

117 Suchyta MR, Clemmer TP, Orme Jr JF et al. Increased survival of ARDS patients with severe hypoxemia (ECMO criteria). Chest 1991; 99 (4): 951-955.

118 Ashbaugh D, Bigelow DB, Petty T et al. Acute respiratory distress in adults. The Lancet 1967; 290 (7511): 319-323.

119 Bernard GR, Artigas A, Brigham KL et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. American journal of respiratory and critical care medicine 1994; 149 (3): 818-824.

120 Force ADT, Ranieri V, Rubenfeld G et al. Acute respiratory distress syndrome. Jama 2012; 307 (23): 2526-2533.

121 Thille AW, Esteban A, Fernández-Segoviano P et al. Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy. American journal of respiratory and critical care medicine 2013; 187 (7): 761-767.

122 Huppert LA, Matthay MA, Ware LB. Pathogenesis of acute respiratory distress syndrome. *Seminars in respiratory and critical care medicine.* 40. Thieme Medical Publishers; 2019:031-039.

123 Wiener-Kronish J, Gropper M, Matthay M. The adult respiratory distress syndrome: definition and prognosis, pathogenesis and treatment. BJA: British Journal of Anaesthesia 1990; 65 (1): 107-129.

124 Matthay M. The adult respiratory distress syndrome. New insights into diagnosis, pathophysiology, and treatment. The Western Journal of Medicine 1989; 150 (2): 187-194.

125 Chen-Yoshikawa TF. Ischemia–Reperfusion Injury in Lung Transplantation. Cells 2021; 10 (6): 1333.

126 Liu X, Cao H, Li J et al. Autophagy induced by DAMPs facilitates the inflammation response in lungs undergoing ischemia-reperfusion injury through promoting TRAF6 ubiquitination. Cell Death & Differentiation 2017; 24 (4): 683-693.

127 Hidalgo M, Sarathchandra P, Fryer P et al. Scanning electron microscopic changes in morphology of pulmonary endothelium in rat lung isografts following hypothermic ischaemic storage and transplantation. International journal of experimental pathology 1995; 76 (5): 339.

128 Hidalgo M, Shah K, Fuller B et al. Cold ischemia–induced damage to vascular endothelium results in permeability alterations in transplanted lungs. The Journal of thoracic and cardiovascular surgery 1996; 112 (4): 1027-1035.

129 Chatterjee S, Nieman GF, Christie JD et al. Shear stress-related mechanosignaling with lung ischemia: lessons from basic research can inform lung transplantation. American Journal of Physiology-Lung Cellular and Molecular Physiology 2014; 307 (9): L668-L680.

130 Sun H, Deng M, Chen W et al. Graft dysfunction and rejection of lung transplant, a review on diagnosis and management. The Clinical Respiratory Journal 2022; 16 (1): 5-12.

131 Choi JK, Kearns J, Palevsky HI et al. Hyperacute rejection of a pulmonary allograft: immediate clinical and pathologic findings. American journal of respiratory and critical care medicine 1999; 160 (3): 1015-1018.

132 Glanville AR. Antibody-mediated rejection in lung transplantation: myth or reality? The Journal of heart and lung transplantation 2010; 29 (4): 395-400.

133 Snell GI, Yusen RD, Weill D et al. Report of the ISHLT Working Group on primary lung graft dysfunction, part I: definition and grading—a 2016 Consensus Group statement of the International Society for Heart and Lung Transplantation. The Journal of Heart and Lung Transplantation 2017; 36 (10): 1097-1103.

134 Lee JC, Christie JD. Primary graft dysfunction. Clinics in chest medicine 2011; 32 (2): 279-293.

135 Christie JD, Kotloff RM, Ahya VN et al. The effect of primary graft dysfunction on survival after lung transplantation. American journal of respiratory and critical care medicine 2005; 171 (11): 1312-1316.

136 Shah RJ, Bellamy SL, Localio AR et al. A panel of lung injury biomarkers enhances the definition of primary graft dysfunction (PGD) after lung transplantation. The Journal of heart and lung transplantation 2012; 31 (9): 942-949.

137 Porteous MK, Lee JC. Primary graft dysfunction after lung transplantation. Clinics in Chest Medicine 2017; 38 (4): 641-654.

138 Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. New England Journal of Medicine 2017; 377 (6): 562-572.

139 Dunham-Snary KJ, Wu D, Sykes EA et al. Hypoxic pulmonary vasoconstriction: from molecular mechanisms to medicine. Chest 2017; 151 (1): 181-192.

140 Van Slambrouck J, Van Raemdonck D, Vos R et al. A Focused Review on Primary Graft Dysfunction after Clinical Lung Transplantation: A Multilevel Syndrome. Cells 2022; 11 (4): 745.

141 Kreisel D, Krupnick AS, Puri V et al. Short-and long-term outcomes of 1000 adult lung transplant recipients at a single center. The Journal of thoracic and cardiovascular surgery 2011; 141 (1): 215-222.

142 Roden AC, Aisner DL, Allen TC et al. Diagnosis of acute cellular rejection and antibody-mediated rejection on lung transplant biopsies: a perspective from members of the pulmonary pathology society. Archives of Pathology & Laboratory Medicine 2017; 141 (3): 437-444.

143 Stewart S, Fishbein MC, Snell GI et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. The Journal of heart and lung transplantation 2007; 26 (12): 1229-1242.

144 Todd JL, Neely ML, Kopetskie H et al. Risk factors for acute rejection in the first year after lung transplant. A multicenter study. American journal of respiratory and critical care medicine 2020; 202 (4): 576-585.

145 Verleden GM, Raghu G, Meyer KC et al. A new classification system for chronic lung allograft dysfunction. 33. Elsevier; 2014:127-133.

146 Welsh CH, Wang TS, Lyu DM et al. An international ISHLT/ATS/ERS clinical practice guideline: summary for clinicians. Bronchiolitis obliterans syndrome complicating lung transplantation. Annals of the American Thoracic Society 2015; 12 (1): 118-119.

147 Abdoul N, Legeai C, Cantrelle C et al. Impact of ex vivo lung perfusion on brain-dead donor lung utilization: The French experience. Am J Transplant 2022; 22 (5): 1409-1417.

148 Estenne M, Hertz MI. Bronchiolitis obliterans after human lung transplantation. American journal of respiratory and critical care medicine 2002; 166 (4): 440-444.

149 Pinezich M, Vunjak-Novakovic G. Bioengineering approaches to organ preservation ex vivo. Experimental Biology and Medicine 2019; 244 (8): 630-645.

150 Hardesty RL, Griffith BP. Autoperfusion of the heart and lungs for preservation during distant procurement. The Journal of thoracic and cardiovascular surgery 1987; 93 (1): 11-18.

151 Steen S, Ingemansson R, Eriksson L et al. First human transplantation of a nonacceptable donor lung after reconditioning ex vivo. The annals of thoracic surgery 2007; 83 (6): 2191-2194.

152 Ingemansson R, Eyjolfsson A, Mared L et al. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. The Annals of thoracic surgery 2009; 87 (1): 255-260.

153 Cypel M, Rubacha M, Yeung J et al. Normothermic ex vivo perfusion prevents lung injury compared to extended cold preservation for transplantation. American Journal of Transplantation 2009; 9 (10): 2262-2269.

154 Lindstedt S, Hlebowicz J, Koul B et al. Comparative outcome of double lung transplantation using conventional donor lungs and non-acceptable donor lungs reconditioned ex vivo. Interactive cardiovascular and thoracic surgery 2011; 12 (2): 162-165.

155 Cypel M, Yeung JC, Liu M et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. New England journal of medicine 2011; 364 (15): 1431-1440.

156 Warnecke G, Moradiellos J, Tudorache I et al. Normothermic perfusion of donor lungs for preservation and assessment with the Organ Care System Lung before bilateral transplantation: a pilot study of 12 patients. The Lancet 2012; 380 (9856): 1851-1858.

157 Van Raemdonck D, Rega F, Rex S et al. Machine perfusion of thoracic organs. Journal of thoracic disease 2018; 10 (Suppl 8): S910.

158 Whitson B, Shukrallah B, Mulligan M et al. Ex-vivo lung perfusion in donation after circulatory death lung transplantation increases donor utilization: analysis of the NOVEL extension trial. The Journal of Heart and Lung Transplantation 2018; 37 (4): S147-S148.

159 Erasmus ME, Van Raemdonck D, Akhtar MZ et al. DCD lung donation: donor criteria, procedural criteria, pulmonary graft function validation, and preservation. Transplant International 2016; 29 (7): 790-797.

160 Steen S, Liao Q, Wierup PN et al. Transplantation of lungs from non–heart-beating donors after functional assessment ex vivo. The Annals of thoracic surgery 2003; 76 (1): 244-252.

161 Loor G. EVLP: ready for prime time? *Seminars in Thoracic and Cardiovascular Surgery.* 31. Elsevier; 2019:1-6.

162 Van Raemdonck D, Neyrinck A, Cypel M et al. Ex-vivo lung perfusion. Transplant International 2015; 28 (6): 643-656.

163 Cypel M, Yeung JC, Donahoe L et al. Normothermic ex vivo lung perfusion: does the indication impact organ utilization and patient outcomes after transplantation? The Journal of Thoracic and Cardiovascular Surgery 2020; 159 (1): 346-355. e341.

164 Reeb J, Cypel M. Ex vivo lung perfusion. Clinical transplantation 2016; 30 (3): 183-194.

165 Aigner C, Slama A, Hötzenecker K et al. Clinical ex vivo lung perfusion—pushing the limits. American journal of transplantation 2012; 12 (7): 1839-1847.

166 Bennett DT, Reece TB, Smith PD et al. Ex vivo lung perfusion allows successful transplantation of donor lungs from hanging victims. The Annals of thoracic surgery 2014; 98 (3): 1051-1056.

167 Andreasson A, Karamanou DM, Perry JD et al. The effect of ex vivo lung perfusion on microbial load in human donor lungs. The Journal of Heart and Lung Transplantation 2014; 33 (9): 910-916.

168 Zych B, Popov AF, Stavri G et al. Early outcomes of bilateral sequential single lung transplantation after ex-vivo lung evaluation and reconditioning. The Journal of heart and lung transplantation 2012; 31 (3): 274-281.

169 Cypel M, Aigner C, Sage E et al. Three center experience with clinical normothermic ex vivo lung perfusion. The Journal of Heart and Lung Transplantation 2013; 32 (4): S16.

170 Sanchez P, Davis R, D'ovidio F et al. Normothermic ex vivo lung perfusion as an assessment of marginal donor lungs–the NOVEL lung trial. The Journal of Heart and Lung Transplantation 2013; 32 (4): S16-S17.

171 Sanchez P, Davis R, D'Ovidio F et al. The NOVEL lung trial one-year outcomes. The Journal of Heart and Lung Transplantation 2014; 33 (4): S71-S72.

172 Fisher A, Andreasson A, Chrysos A et al. An observational study of Donor Ex Vivo Lung Perfusion in UK lung transplantation: DEVELOP-UK. Health Technology Assessment 2016; 20 (85): 1-276.

173 Yeung JC, Krueger T, Yasufuku K et al. Outcomes after transplantation of lungs preserved for more than 12 h: a retrospective study. The Lancet Respiratory Medicine 2017; 5 (2): 119-124.

174 Loor G, Warnecke G, Villavicencio MA et al. Portable normothermic ex-vivo lung perfusion, ventilation, and functional assessment with the Organ Care System on donor lung use for transplantation from extended-criteria donors (EXPAND): a single-arm, pivotal trial. The Lancet Respiratory Medicine 2019; 7 (11): 975-984.

175 Mallea JM, Hartwig MG, Keller CA et al. Remote ex vivo lung perfusion at a centralized evaluation facility. The Journal of Heart and Lung Transplantation 2022; 41 (12): 1700-1711.

176 Wierup P, Haraldsson Å, Nilsson F et al. Ex vivo evaluation of nonacceptable donor lungs. The Annals of thoracic surgery 2006; 81 (2): 460-466.

177 Moradiellos F, Naranjo J, Córdoba M et al. 90 Clinical lung transplantation after ex vivo evaluation of uncontrolled non heart-beating donors lungs: initial experience. The Journal of Heart and Lung Transplantation 2011; 30 (4): S38.

178 Cypel M, Yeung JC, Machuca T et al. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. The Journal of thoracic and cardiovascular surgery 2012; 144 (5): 1200-1207.

179 Wigfield C, Cypel M, Yeung J et al. Successful emergent lung transplantation after remote ex vivo perfusion optimization and transportation of donor lungs. American Journal of Transplantation 2012; 12 (10): 2838-2844.

180 Valenza F, Rosso L, Gatti S et al. Extracorporeal lung perfusion and ventilation to improve donor lung function and increase the number of organs available for transplantation. *Transplantation proceedings.* 44. Elsevier; 2012:1826-1829.

181 Dark J, Karamanou D, Clark S et al. 323 successful transplantation of unusable donor lungs using ex-vivo lung perfusion: the newcastle experience. The Journal of Heart and Lung Transplantation 2012; 31 (4): S115.

182 Hopkins P, Chambers D, Naidoo R et al. Australia's experience with ex-vivo lung perfusion of highly marginal donors. The Journal of Heart and Lung Transplantation 2013; 32 (4): S154.

183 Wallinder A, Ricksten S-E, Silverborn M et al. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case–control study. European Journal of Cardio-Thoracic Surgery 2014; 45 (1): 40-45.

184 Boffini M, Ricci D, Bonato R et al. Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with ex vivo lung perfusion. European Journal of Cardio-Thoracic Surgery 2014; 46 (5): 789-793.

185 Fildes JE, Archer LD, Blaikley J et al. Clinical outcome of patients transplanted with marginal donor lungs via ex vivo lung perfusion compared to standard lung transplantation. Transplantation 2015; 99 (5): 1078-1083.

186 Tikkanen JM, Cypel M, Machuca TN et al. Functional outcomes and quality of life after normothermic ex vivo lung perfusion lung transplantation. The Journal of heart and lung transplantation 2015; 34 (4): 547-556.

187 Bozso S, Vasanthan V, Luc JG et al. Lung transplantation from donors after circulatory death using portable ex vivo lung perfusion. Canadian respiratory journal 2015; 22 (1): 47-51.

188 Mohite P, Maunz O, Popov A et al. Utilization of the organ care system as ex-vivo lung perfusion after cold storage transportation. Perfusion 2015; 30 (8): 698-700.

189 Machuca T, Mercier O, Collaud S et al. Lung transplantation with donation after circulatory determination of death donors and the impact of ex vivo lung perfusion. American Journal of Transplantation 2015; 15 (4): 993-1002.

190 Wallinder A, Riise GC, Ricksten S-E et al. Transplantation after ex vivo lung perfusion: a midterm follow-up. The Journal of Heart and Lung Transplantation 2016; 35 (11): 1303-1310.

191 Zeriouh M, Sabashnikov A, Mohite PN et al. Utilization of the organ care system for bilateral lung transplantation: preliminary results of a comparative study. Interactive cardiovascular and thoracic surgery 2016; 23 (3): 351-357.

192 Slama A, Schillab L, Barta M et al. Standard donor lung procurement with normothermic ex vivo lung perfusion: a prospective randomized clinical trial. The Journal of Heart and Lung Transplantation 2017; 36 (7): 744-753.

193 Luc J, Jackson K, Weinkauf J et al. Feasibility of lung transplantation from donation after circulatory death donors following portable ex vivo lung perfusion: a pilot study. *Transplantation proceedings.* 49. Elsevier; 2017:1885-1892.

194 Warnecke G, Van Raemdonck D, Smith MA et al. Normothermic ex-vivo preservation with the portable Organ Care System Lung device for bilateral lung transplantation (INSPIRE): a randomised, open-label, non-inferiority, phase 3 study. The Lancet Respiratory Medicine 2018; 6 (5): 357-367.

195 Koch A, Pizanis N, Olbertz C et al. One-year experience with ex vivo lung perfusion: Preliminary results from a single center. The International Journal of Artificial Organs 2018; 41 (8): 460-466.

196 Divithotawela C, Cypel M, Martinu T et al. Long-term outcomes of lung transplant with ex vivo lung perfusion. JAMA surgery 2019; 154 (12): 1143-1150.

197 Nilsson T, Wallinder A, Henriksen I et al. Lung transplantation after ex vivo lung perfusion in two Scandinavian centres. European Journal of Cardio-Thoracic Surgery 2019; 55 (4): 766-772.

198 Ghaidan H, Fakhro M, Andreasson J et al. Ten year follow-up of lung transplantations using initially rejected donor lungs after reconditioning using ex vivo lung perfusion. Journal of Cardiothoracic Surgery 2019; 14 (1): 1-8.

199 Zhang ZL, van Suylen V, van Zanden JE et al. First experience with ex vivo lung perfusion for initially discarded donor lungs in the Netherlands: a single-centre study. Eur J Cardiothorac Surg 2019; 55 (5): 920-926.

200 Fumagalli J, Rosso L, Gori F et al. Early pulmonary function and mid-term outcome in lung transplantation after ex-vivo lung perfusion - a single-center, retrospective, observational, cohort study. Transpl Int 2020; 33 (7): 773-785.

201 Leiva-Juarez MM, Urso A, Arango Tomas E et al. Extended post-ex vivo lung perfusion cold preservation predicts primary graft dysfunction and mortality: Results from a multicentric study. J Heart Lung Transplant 2020; 39 (9): 954-961.

202 Okahara S, Levvey B, McDonald M et al. Common Criteria for Ex Vivo Lung Perfusion Have No Significant Impact on Posttransplant Outcomes. Ann Thorac Surg 2021; 111 (4): 1156-1163.

203 Undurraga MF, Vega SJ, Fajardo JC et al. [Lung transplant with ex vivo lung perfusion, Chilean and Latin American first experience]. Rev Med Chil 2021; 149 (2): 171-177.

204 Peel JK, Keshavjee S, Naimark D et al. Determining the impact of ex-vivo lung perfusion on hospital costs for lung transplantation: A retrospective cohort study. J Heart Lung Transplant 2022.

205 Franco NH. Animal experiments in biomedical research: a historical perspective. Animals 2013; 3 (1): 238-273.

206 Brun-Buisson C, Minelli C, Bertolini G et al. Epidemiology and outcome of acute lung injury in European intensive care units. Intensive care medicine 2004; 30 (1): 51-61.

207 Ferdinand JR, Hosgood SA, Moore T et al. Cytokine absorption during human kidney perfusion reduces delayed graft function–associated inflammatory gene signature. American Journal of Transplantation 2021; 21 (6): 2188-2199.

208 Ankawi G, Xie Y, Yang B et al. What have we learned about the use of cytosorb adsorption columns? Blood purification 2019; 48 (3): 196-202.

209 Matute-Bello G, Downey G, Moore BB et al. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. American journal of respiratory cell and molecular biology 2011; 44 (5): 725-738.

210 Schindelin J, Arganda-Carreras I, Frise E et al. Fiji: an open-source platform for biological-image analysis. Nature methods 2012; 9 (7): 676-682.

211 Park WY, Goodman RB, Steinberg KP et al. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. American journal of respiratory and critical care medicine 2001; 164 (10): 1896-1903.

212 Meduri GU, Headley S, Kohler G et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS: plasma IL-1β and IL-6 levels are consistent and efficient predictors of outcome over time. Chest 1995; 107 (4): 1062-1073.

213 Bauer TT, Montón C, Torres A et al. Comparison of systemic cytokine levels in patients with acute respiratory distress syndrome, severe pneumonia, and controls. Thorax 2000; 55 (1): 46-52.

214 Ballard-Croft C, Wang D, Sumpter LR et al. Large-animal models of acute respiratory distress syndrome. The Annals of thoracic surgery 2012; 93 (4): 1331-1339.

215 Iskender I, Cosgun T, Arni S et al. Cytokine filtration modulates pulmonary metabolism and edema formation during ex vivo lung perfusion. The Journal of Heart and Lung Transplantation 2018; 37 (2): 283-291.

216 Bannerman DD, Goldblum SE. Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. American Journal of Physiology-Lung Cellular and Molecular Physiology 2003; 284 (6): L899-L914.

217 Kradin RL, Digumarthy S. The pathology of pulmonary bacterial infection. *Seminars in diagnostic pathology*. 34. 2017:498-509.

218 Butt Y, Kurdowska A, Allen TC. Acute lung injury: a clinical and molecular review. Archives of Pathology and Laboratory Medicine 2016; 140 (4): 345-350.

219 Popescu M, Dima S, David C et al. Standard renal replacement therapy combined with hemoadsorption in the treatment of critically ill septic patients. Therapeutic Apheresis and Dialysis 2021; 25 (5): 663-670.

220 Friesecke S, Stecher S-S, Gross S et al. Extracorporeal cytokine elimination as rescue therapy in refractory septic shock: a prospective single-center study. Journal of Artificial Organs 2017; 20 (3): 252-259.

221 Fakhro M, Ingemansson R, Algotsson L et al. Impact of forced expiratory volume in 1 second (FEV1) and 6-minute walking distance at 3, 6, and 12 months and annually on survival and occurrence of bronchiolitis obliterans syndrome (BOS) after lung transplantation. Annals of Transplantation 2017; 22: 532-540.

222 Mulloy DP, Stone ML, Crosby IK et al. Ex vivo rehabilitation of non–heart-beating donor lungs in preclinical porcine model: delayed perfusion results in superior lung function. The Journal of thoracic and cardiovascular surgery 2012; 144 (5): 1208-1216.

223 Nakajima D, Chen F, Yamada T et al. Reconditioning of lungs donated after circulatory death with normothermic ex vivo lung perfusion. The Journal of heart and lung transplantation 2012; 31 (2): 187-193.

224 Riddell P, Egan JJ. Will ex-vivo lung perfusion allow a move towards daytime lung transplantation surgery? The Lancet Respiratory medicine 2017; 5 (2): 88-89.

225 Iskender I, Cypel M, Martinu T et al. Effects of warm versus cold ischemic donor lung preservation on the underlying mechanisms of injuries during ischemia and reperfusion. Transplantation 2018; 102 (5): 760-768.

226 Novick RJ, Bennett LE, Meyer DM et al. Influence of graft ischemic time and donor age on survival after lung transplantation. The Journal of heart and lung transplantation 1999; 18 (5): 425-431.

227 Clavien P-A, Harvey PR, Strasberg SM. Preservation and reperfusion injuries in liver allografts. Transplantation 1992; 53 (5): 957-978.

228 Thabut G, Mal H, Cerrina J et al. Graft ischemic time and outcome of lung transplantation: a multicenter analysis. American journal of respiratory and critical care medicine 2005; 171 (7): 786-791.

229 Chrousos GP. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. New England Journal of Medicine 1995; 332 (20): 1351-1363.

230 Karalis K, Muglia LJ, Bae D et al. CRH and the immune system. Journal of neuroimmunology 1997; 72 (2): 131-136.

231 Webster EL, Torpy DJ, Elenkov IJ et al. Corticotropin-releasing hormone and inflammation. Annals of the New York Academy of Sciences 1998; 840 (1): 21-32.

232 Vale W, Spiess J, Rivier C et al. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science 1981; 213 (4514): 1394-1397.

233 Nezi M, Mastorakos G, Mouslech Z. Corticotropin releasing hormone and the immune/inflammatory response. 2015.

234 Baigent S. Peripheral corticotropin-releasing hormone and urocortin in the control of the immune response. Peptides 2001; 22 (5): 809-820.

235 Silverman ES, Breault DT, Vallone J et al. Corticotropin-releasing hormone deficiency increases allergen-induced airway inflammation in a mouse model of asthma. Journal of Allergy and Clinical Immunology 2004; 114 (4): 747-754.

236 Curley GF, Hayes M, Ansari B et al. Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat. Thorax 2012; 67 (6): 496-501.

237 Devaney J, Horie S, Masterson C et al. Human mesenchymal stromal cells decrease the severity of acute lung injury induced by E. coli in the rat. Thorax 2015; 70 (7): 625-635.

238 Hayes M, Masterson C, Devaney J et al. Therapeutic efficacy of human mesenchymal stromal cells in the repair of established ventilator-induced lung injury in the rat. Anesthesiology 2015; 122 (2): 363-373.

239 Gramatté J, Pietzsch J, Bergmann R et al. Causative treatment of acid aspiration induced acute lung injury–Recent trends from animal experiments and critical perspective. Clinical Hemorheology and Microcirculation 2018; 69 (1-2): 187-195.

240 Raghavendran K, Nemzek J, Napolitano LM et al. Aspiration-induced lung injury. Critical care medicine 2011; 39 (4): 818.

241 Hu X, Lee JS, Pianosi PT et al. Aspiration-related pulmonary syndromes. Chest 2015; 147 (3): 815-823.

242 Price LC, McAuley DF, Marino PS et al. Pathophysiology of pulmonary hypertension in acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology 2012; 302 (9): L803-L815.

243 Chaney J, Suzuki Y, Cantu III E et al. Lung donor selection criteria. Journal of thoracic disease 2014; 6 (8): 1032.

244 Mariscal A, Caldarone L, Tikkanen J et al. Pig lung transplant survival model. Nature protocols 2018; 13 (8): 1814-1828.

Clinical and Preclinical lung transplantation in the aspects of improving outcome

HAIDER GHAIDAN graduated with an MD from Basrah University, Iraq in 1995, and he began his residency in cardiac surgery in 1998 at Saddam center for cardiac surgery in Baghdad, Iraq. He moved to Libya and qualified as a cardiac surgeon at National Heart Center, Tripoli. After moving to Sweden, he completed his training and became qualified in cardiothoracic surgery under the Swedish National Board of Health in 2014. In April 2015, he began his employment as a Consultant Cardiac Surgeon at the Thoracic Centre, Karlskrona Hospital, Sweden.

In April 2017, he began his present employment as a Consultant Thoracic Surgeon at the Department of Cardiothoracic Surgery, Skåne University Hospital, Lund, Sweden. In 2017 he commenced his PhD studies.

Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Cardiothoracic Surgery
Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2023:30
ISBN 978-91-8021-369-1
ISSN 1652-8220