Hypoxic Pulmonary Vasoconstriction Is a More Accurate Parameter Than P/F Ratio to Measure Lung Function on Ex-Vivo Lung Perfusion

BY

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Abstract

Background: Rates of thoracic donor organ utilization remains very low at 15-25% internationally. Development of normothermic Ex-Vivo Lung Perfusion (EVLP) has shown to increase rates of donor lung utilization. Adoption of EVLP to routine clinical practice will require more sensitive physiological parameters of lung function on EVLP. Currently, P/F ratio has been used as the most reliable parameter of lung function on EVLP. Our data suggests a change in the lungs response to hypoxic pulmonary vasoconstriction (HPV) is a more sensitive parameter to detect early lung dysfunction on EVLP.

Methods: Our novel EVLP platform as an apparatus for large animal model and human pre-clinical/clinical investigation, has two centrifugal flow pumps (instead of one) and it is equipped with a computer-controlled feedback system that provides real-time control of PA and LA pressures/flows as well as monitoring/recording the ventilator parameters (such as peak/plateau pressures, PEEP) and lung perfusion data (i.e. PVR) with 10sec intervals. We challenged the lungs with hypoxia serially during the EVLP run by ventilating the lungs with 100% N2 to observe the response of hypoxic pulmonary vasoconstriction (HPV).

Results: We performed EVLP on 10 domestic pigs (40-45kg) for 12 hours each. We found our device provided excellent lung function while on EVLP up to 12 hours, with the P/F ratio remaining >400 mmHg throughout. EVLP was maintained at 75% cardiac output (2.2-2.4 L/min), where PA pressure and LA pressure are controlled constant by the device software at 8 mmHg and 2 mmHg, respectively. Lung compliance remained stable up to 12 hours without significant changes to peak/mean airway pressures, and PVR. When lungs were challenged with hypoxia, there was a serial blunting in HPV response over the course of 12 hours with a decreased rise in PVR when challenged with hypoxia ($p<0.05$).

Conclusions: As experience is gained with EVLP, appreciation of the most sensitive parameters for donor lung function is critical to allow for extended EVLP runs, and to assess therapeutic interventions. The conventional use of P/F ratios may not reflect sub-clinical lung dysfunction, while response to HPV may be a more sensitive physiologic measurement of lung function.

Acronyms:

EVLP: Ex-vivo lung perfusion.

P/F ratio: Pulmonary venous PO2/Fraction of inhaled oxygen.

HPV: Hypoxic Pulmonary Vasoconstriction

Preface

This thesis is an original work by Almothana F. Alzamil. The research project, on which this thesis is based, received research ethical approval from the Animal Care and Use Committee for Health Science in University of Alberta, Project name “Ex-vivo cardiothoracic organ perfusion”, No. AUP00000943, June 21, 2014

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To my deeply missed grandmother, I dedicate my thesis

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List of Abbreviations:

A1AD	Alpha 1 anti-trypsin deficiency
AMPK	Adenosine monophosphate kinase
CMV	Cytomegalo virus
CO2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CF	Cystic fibrosis
ELISA	Enzyme-linked immunosorbent assay
ESLD	End stage lung disease
EVLP	Ex-vivo Lung Perfusion
FiO2	Fraction of inspired oxygen
HPV	Hypoxic Pulmonary Vasoconstriction
ILD	Interstitial lung disease
LA	Left atrium
O2	Oxygen
OCS	Organ Care System
PA	Pulmonary artery
PaCo2	Arterial Partial pressure of Carbon dioxide
PaO2	Arterial Partial pressure of oxygen
P/F ratio	Pulmonary venous PO2/Fraction of inhaled oxygen.
PGD	Primary graft dysfunction
PVR	Pulmonary vascular resistance
QOL	Quality of life
TNF-α	Tumor necrosis factor alpha (TNF-α)

1 Chapter 1

1.1 Overview:

The University of Alberta has one of the largest lung transplant centers in Canada. Alberta lung transplant program covers a catchment population of more than 7 million Canadians in the provinces of Alberta, Northern British Columbia and Saskatchewan (**Figure 1**). Among all the provinces of the country, Alberta has the lowest organ donation rate which affect the chances of patients on the lung transplant waitlist to receive a transplant. Another unique challenge to the thoracic transplant center at the University of Alberta is being the most geographically isolated program in North America and Europe which makes the procurement of donor transplant organ challenging(1).

Lung transplantation is the only lifesaving treatment for patients with end stage lung disease as it has been shown to increase the survival rate for those patients following lung transplantation(2). Additionally, more than one study on patient's health status, functional status and adjustment after lung transplantation have demonstrated a significant improvement in quality of life (QOL) outcomes following lung transplantation comparing to the QOL before receiving a lung transplant(3)(4)(5).

So experiments taking place in our laboratory have been focused on helping one of the most vulnerable patient population in medicine, patients with chronic end stage lung disease (ESLD) (6). Our overall objective is to increase the pool of donor thoracic organ

and increase the rate of organ utilization by using normothermic ex-vivo lung perfusion (EVLP). EVLP is a technology that gives us the opportunity to assess, evaluate and repair donor lungs outside of the body. This permit both reduce ischemia time caused by long distance of available donor lung and repair marginal donors before transplant, this will lead to a positive impact for patients on lung transplantation wait list.

1.2 Embryology of the lung:

Lungs start to develop at week 3 of embryogenesis, when a groove start to appear in the ventral floor of the foregut. This depression (or groove) becomes an out pouching of endoderm and splanchnic mesoderm. Endoderm is the origin of the epithelium and glands while mesoderm becomes cartilage, muscular components and connective tissue (7)(8).

At the Embryonic period (gestation week 3 to 7), two lung buds will develop (9) and will undergo multiple branching processes to form trachea and main bronchi (9). It begins with the growth of the right main bronchi and the left main bronchi, then trachea will separates from esophagus, after that the formation of the primordial bronchial tree. By the 17th week of gestation 70% of airways is formed and the bronchi resemble tubular glands, this stage is called as (pseudoglandular stage). After that, three other stages of lung development will follow: Canalicular stage (between 17-26 weeks) where vascular system and gas exchange are (air-blood barrier) formed, Terminal sac formation (from 24th week to birth) and alveoli formation which first appear at week 20 of gestation and continue developing up to the age of 8-10 years old (7)(8).

The pulmonary vasculature formation starts early at the embryonic period even before the formation of lung morphology (7). Although lung vascular growth and development have been studied, still the full picture of this process is not yet clear(10). The formation of pulmonary circulation goes through multiple branching and outgrowths changes during different stages of lung development. Two basic processes are known to take place in pulmonary vascular growth and development: vasculogenesis, which is new blood vessels formation from endothelial cells within the immature mesenchyme and angiogenesis, which is the growth of new blood vessels from pre-existing vessels (10).

The multiple branching of lung components (airways and vessels) will lead to the formation of multiple bronchopulmonary segments and each segment has its own arterial supply, venous drainage and bronchus, So because of that each bronchopulmonary segment can act individually (7).

By the end of gestation, two lungs will form with five well-defined lobes, two on the left (upper and lower lobes) and three on the right side (upper, middle and lower lobes) (11)(12).

1.3 Respiratory system anatomy

1.3.1 Anatomy of the Lung:

Each lung formed of more than one lobe, three lobes on the right lung and two lobes on the left lung in addition to the lingula which resemble the middle lobe of the right

lung. Lobes are separated by two fissures: The major fissure, which separates the lower lobe from the upper and middle lobes and the minor fissure, which separates the upper lobe from the middle lobe. Each lobe is composed of bronchopulmonary segments that function individually and contain their own bronchus, arterial supply and venous drainage, because of this segment can be removed without an effect on other segments function. The right lung consist of 10 segments: three segments in the upper lobe (apical, posterior and anterior), two segments in the middle lobe (lateral and medial), and five segments in the lower lobe (superior, medial basal, anterior basal, lateral basal and posterior basal). While the left lung consist of eight segments. Four segments in the upper lobe (apical posterior, anterior, superior lingular and inferior lingular), four segments in the lower lobe (superior, anteromedial basal, lateral basal and posterior basal) (7). Long narrow bronchus is a unique anatomical feature in the middle lobe which makes it more susceptible to chronic inflammatory changes due to bronchial compression caused by lymph nodes or masses, and these inflammatory changes called "middle lobe syndrome" (13) .

The average weight of the lung is around 900-1000 g, around 40% of the weight is blood (14)(15). The gas volume depend on the status of the respiration, so it's around 2.5 L at end expiration, whereas at maximal inspiration it may reach up to 6 L (16).

1.3.2 Anatomy of the Conducting airways:

The airways play a major role in lung function since they are responsible for conducting the outside air to the terminal respiratory units, and understanding airways

function and anatomy will help in better understanding of normal lung function, mechanism of some lung diseases and subsequent abnormal lung function.

Starting with the trachea which is considered to be the most part in the respiratory system that is exposed to the environmental factors. It's a cartilage rigid tube with C-shaped rings which the opening side facing posteriorly and covered by trachealis muscle. The shape of the trachea will protect it from frontal injury and when there is a negative change in intrathoracic pressure during respiration it will prevent collapsing. In addition to that the trachea will function by moisturising and warming the inspired air, and prevent dust particles to reach the terminal bronchopulmonary tree, it does all that by the respiratory epithelium, sub mucous gland and smooth muscle. So any disease to the trachea it will affect the lung function(8).

The bronchi starts at the bifurcation of the trachea (carina). It is cartilaginous in nature and with (C-shaped) rings in the main bronchi then it will change to puzzle piece-like plates in the lung parenchyma.

The bronchioles or the membranous bronchioles are noncartilaginous, small diameter and short airways. when lung volume increases they passively enlarge because they are embedded in the connective tissue of the lungs (17). They don't have alveoli in their walls in turn they direct air to alveoli(8).

1.3.3 Terminal respiratory units:

The terminal respiratory unit consist of: respiratory bronchiole, alveolar duct and the alveoli. Structural and functional existence was first described by Hayek(18). There are around 100 alveolar ducts and 2000 alveoli in each terminal respiratory unit. Normal adult lungs contain around 150,000 terminal respiratory units (19)(20)(21).

The terminal respiratory units have a very important functional role in the respiratory system. It's responsible for gas phase diffusion in other words it allows the Oxygen (O_2) and Carbon dioxide (CO_2) inhaled and transported by the bronchial tree to the alveoli to diffuse between the blood and the inhaled air across the respiratory membranes which is formed between the alveoli and capillary wall (16,16).

1.3.4 Types of alveolar cells

Alveolar epithelium has more than one type of cells that can be seen by light and electron microscope. These cells are: Type 2 cells, which is small in size and cuboidal in shape and Type 1 cells, large and flattened cells, they occupied a very large alveolar surface area (90%) comparing to the small alveolar surface area occupied by Type 2 cells. However, Type 2 cells are more in quantity comparing to Type 1 cells (22). Both types have different cells structure and therefore different functions in the respiratory system.

Type 1 cell is composed of a centrally located nucleus and a large cytoplasmic process which will form a large surface area for gas exchange. However, the large surface area of

the cytoplasmic processes may cause a problem in transportation of new proteins(16). They contain a small variable number of intracytoplasmic vesicles which may have physiologic significance(23). Some biochemical analyses showed that these vesicles contain Cavolin protein(24)(25). Caveolin is a protein that bind to free cholesterol and regulate the efflux of cholesterol when the concentration rise intracellularly(26).Type 1 alveolar cells have a role in gas exchange by being responsible for providing thin cellular barrier for gas exchange, furthermore, these cells are responsible for some proteins expression. Unlike Type 2 cells, Type1 cells can't proliferate by its self and they rely on Type 2 cells for their proliferation (27).

Type 2 alveolar cells are small cuboidal cells with short apical Microvillus. Normally Type 2 cells attach tightly to neighboring Type 1 cells to form impermeable seal between alveolar air and connective tissue spaces(16,16).

The distinguishing feature of Type 2 cell is having intracellular lamellar bodies. These granules contain pulmonary surfactant and composed of phospholipids species similar to those of lavaged surfactant(28). The granules also include various proteins such as surfactant proteins , lysosomal enzymes, H+ transporter and other molecules(29)(30).

Type 2 cell is the major synthesizing and secreting factory of the alveolar epithelium, it implements epithelial repair by the ability to proliferate. Types of proteins produced by type 2 cell includes surfactant associated proteins, adsorption of surfactant lipids, immunomodulatory functions, receptor for several growth factors and enzymes. These

cells can generate both type 1 cell and type 2 cell. However, Type 2 cells functions are more known than type 1 cells and this is due to successful methods for isolating a large number and high purity of human type 2 cells for function and response studies(31)(32). Type 1 cells have been isolated as well to more study their function(33)(34).

1.3.5 Anatomy of pulmonary circulation

The pulmonary arteries carry the venous blood to the alveolar spaces in the lungs for oxygenation and gas exchange. The entrance of the pulmonary artery circulation to the lungs is through the hilum and close to the main bronchus. The conus arteriosus of the right ventricle of the heart will give rise to the pulmonary arteries which will run in parallel with the airways through the lung and this will keep us reminding the relation between perfusion and ventilation and to evaluate the efficiency of normal lung function (35)(36). The bifurcation of the main pulmonary artery into left and right trunks that follow the right and left main bronchi into the lung will take place at the forth thoracic vertebral body. Comparing the pulmonary circulation to the systemic circulation, it is considered to be shorter in length and a lower pressure system than the systemic circulation(37)(38). The diameter of the airway and the parallel pulmonary artery is almost the same. As the systemic vessels, the pulmonary arteries have three layers: intima, media and the adventitia however, the muscular layer is thicker in systemic vessels(8).

The pulmonary veins bud bed serves as a capacitance reservoir between the right and the left sides of the heart(16). So they carry oxygenated blood from the lungs back to the

heart to be distributed by systemic circulation. Unlike pulmonary arteries, pulmonary veins travel in separate course in the interlobular septa and they don't travel beside airways. So this makes pulmonary veins hard to identify (35)(39). To facilitate finding and locating a pulmonary vein you have to find the junction of the pleura with an interlobular septum and it's important for systemic evaluation with every lung biopsy in patient with diffuse lung disease(8).

In normal lung, and because of increased luminal pressure (at the bottom of the lung) that lead to expansion of blood vessels and increase their volume, the blood volume is greater at the bottom of the lung. Pulmonary blood vessels pressure is different according to their level in the lung, so vessels near to the top of the lung will have less pressure while vessels near to the bottom of the lung will have higher pressure.

Any segment of pulmonary vessels can contribute to active vasomotion (40), because smooth muscle can be found in the arterial and the venous pulmonary vessels in addition to pre and post capillary vessels (41)(42). Vasoactivity plays a major role in the local regulation of blood flow in relation to ventilation (43). Extension of vascular smooth muscle to the capillary level may occur in pathologic conditions (44)(45). Endothelial cells founded in the pulmonary vasculature play a major role in regulating vascular tone and reactivity. They are indicator for numbers of metabolic activities(16). They are as important as endothelial cells of other organs but what makes endothelial cells of pulmonary vasculature with greater responsibility and much more importance than in other organs is the central position of the lung were the entire cardiac output passes

(46)(47). The expression of endothelial nitric oxide synthase will generate a potent vasodilator (nitric oxide) locally in the lung which is important for regulation of blood flow (48) (49). In order to have the role of the endothelial cells in regulation of vascular tone and reactivity there should be a direct contact between pulmonary endothelial cells in small arteries and veins and smooth muscle cells surrounding them. Such contacts had been described in the lungs of small animals(50) (51).

1.4 Gaseous exchange in the alveoli

Gas exchange in the alveoli is the diffusion of gases (Oxygen O_2 and Carbon dioxide CO_2) between the inhaled air and the blood were the gas molecule moves from a higher gas concentration area to a lower gas concentration area until gases in alveoli and capillaries area reaches to an equilibrium. Factors other than pressure gradients that can have an influence on the diffusion process of gaseous across the Alveolar-Capillary membrane are: thickness of the membrane, solubility and diffuse ability of a gas through liquid. The alveolar-capillary membrane is formed of: fluid layer of the alveolus, alveolar epithelium, alveolar basement membrane, interstitial space, capillary basement membrane, capillary endothelium and the plasma in the capillary blood.

The partial pressures of O_2 and CO_2 are different in the air, alveoli and blood. The air in the alveoli is highly concentrated with Oxygen (PO_2= 100 mm Hg) but with a lower concentration of carbon dioxide (PCO_2= 40 mm Hg). Conversely, the venous blood passes through the alveolar-capillary membrane has a higher concentration of Carbon

dioxide (PCO_2= 46 mm Hg) than concentration of Oxygen (PO_2= 40 mm Hg). So, Oxygen will diffuse from alveolus through the membrane to the venous blood while Carbon dioxide will diffuse from venous blood to the alveoli until they reach equilibrium (31). Under normal resting conditions, partial pressure of Oxygen and Carbon dioxide reach equilibrium in about 0.25 Seconds but diffusion Of O_2 will be decreased in lungs with pulmonary disease and may not reach equilibrium during exercise or stress (52).

1.5 Lung transplantation

Lung transplantation is the only lifesaving treatment for patients with end stage lung disease as it has been shown to increase the survival rate for those patients following lung transplantation(2). Additionally, more than one study on patient's health status, functional status and adjustment after lung transplantation have demonstrated a significant improvement in quality of life (QOL) outcomes following lung transplantation comparing to the QOL before receiving a lung transplant(3)(4)(5).

1.5.1 Background

The first successful lung transplantation attempt in an animal model was achieved by Metras H, in 1950. Human lung transplantation was first attempted in 1963,by Hardy(53). However, patients who underwent lung transplantation at that era didn't survive long post operatively. It was not until nearly 2 decades later that extended survival was achieved after discovering the benefit of Cyclosporine use post lung transplantation(54). The first successful human single lung transplantation occurred in 1983 and the first double lung

transplantation in 1986 in Toronto(55)(56). Despite the relatively short history of thoracic transplantation, there has been significant improvement in results primarily based on improved preservation solutions, immunosuppression regimes, and specialized patient care by transplant clinics. The Registry of International Society for Heart and Lung Transplantation reported around 50,000 adult lung transplants have been performed around the world between 1985 and 2013 (2). The survival rate for those who underwent primary lung transplantation in this period is 54% at 5 years and the median survival of patients who had Bilateral lung transplantation is 7.1 years vs 4.5 years for those who underwent single lung transplant (2). Additionally, more than one study on patient's health status, functional status and adjustment after lung transplantation have demonstrated a significant improvement in quality of life (QOL) outcomes following lung transplantation comparing to the QOL before receiving a lung transplant (3)(4)(5).

For decades, the gold standard for lung procurement in Canada has been to procure lungs from a donor after flushing them with a low potassium dextran based solution (Perfadex) and transporting them from the donor site at 4-8 ^{o} C, without perfusion or ventilation, back to the recipient hospital(57). Upon arrival at the recipient hospital, donor lungs are then implanted in the recipient. After reimplantation, for the first time after several hours of cold ischemia, perfusion through the pulmonary vasculature and ventilation occur.

Of the solid organ transplants including hearts, livers, and kidneys, the lung transplant patient population has the lowest long-term survival. The primary cause of death in the

first 30 days post lung transplantation is primary graft dysfunction and non-Cytomegalovirus (CMV) infections (2)(6), while from 31 days post lung transplantation to the first year non- CMV virus infections become the most common cause of death. After the first-year post lung transplantation the most common causes of death are: bronchiolitis, non- CMV infections and graft failure.

Primary graft dysfunction (PGD) is defined as an inability of the transplanted lungs to maintain adequate oxygenation because of the diffuse alveolar damage that occur to the transplanted lung as a consequence of ischemia reperfusion injury specially in the first 72 hours post lung transplantation (58). The mechanism of lung injury with primary graft dysfunction and diffuse alveolar damage post lung transplantation is believed to be majorly caused by Ischemic reperfusion injury, as it stimulates the reactive oxygen species (ROS) and activates release of cytokines and chemokines which will propagate an inflammation response that cause injury to the alveoli (59). Signs and symptoms of patients suffering from primary graft dysfunction includes: hypoxia, lung edema and pulmonary infiltrates on chest x ray. Patients with (PGD) have a high chance to develop chronic graft dysfunction in the future. So, preventing primary graft dysfunction is a major concern for all lung transplant surgeon by taken all measures to minimize ischemia time therefore insult caused by primary graft dysfunction to lungs. Currently, variables associated with the length of donor ischemic time include communication and timing between the donor procurement team and the recipient team. The goal is to have the recipient pneumonectomies completed at the time the donor lungs arrive at the recipient

centre, thus allowing for immediate implantation. However, this ideal situation is often times complicated by unexpected factors including, recipient pneumonectomy issues (dense adhesion of lungs), distorted anatomy, potential communication issues arising during donor lung transport, and other unexpected transport delays (weather, and plane complications). As well, the major variable associated with the length of donor ischemic time, at the University of Alberta, is the distance needed to travel to procure donor organs.

1.5.2 Indications for lung transplantation

Chronic obstructive pulmonary disease (COPD) represents the leading indication for lung transplantation and accounts for over (32% of cases) of all procedures performed worldwide (60). Chronic obstructive pulmonary disease (COPD) is a progressive not fully reversible airflow limitation and it's a preventable and treatable disease that may lead to effects not only on the pulmonary system but extra pulmonary effects as well and it is one of the common causes of admissions to the intensive care unit (ICU) (61). The second leading indications is interstitial lung disease (ILD) (24% of cases), then cystic fibrosis (CF; 16% of cases) and Alpha 1 Antitrypsin dysfunction (A1AD 5% of cases) (2). Idiopathic pulmonary arterial hypertension as well was a common indication for transplantation but now with the improvement and advances in medical care and management it is mostly accounts only for around 4% of procedures. Benefits from Lung transplantation for patients who are suffering from lung involvement due to collagen vascular disease (e.g., scleroderma) is still not clear (16).

In contrast, lung transplantation for Lung cancers well known with a High rate of recurrence such as bronchioalveolar carcinoma has been avoided (62)..

1.5.3 Current Method of Lung Donor Procurement:

Since the inception of lung transplantation there has been little change in the methodology of donor lung procurement. In brief, a transplant team will travel to the donor site and clinically assess the donor. This clinical assessment is based on a variety of factors including, arterial blood gases, chest x-rays, and bronchoscopy results. Upon completion of the clinical assessment, a decision as to the suitability of the donor lungs for transplantation is made. If the team renders the donor lungs suitable for transplantation, the procurement procedure is performed. Through a median sternotomy, the donor lungs are exposed and perfused antegrade (via the pulmonary artery) with cold low-potassium Dextran solution (Perfadex) and then perfused retrograde (via the pulmonary veins) with the same solution. The heart is then removed, the airways are inflated to 25 cm of water and clamped, and the double-lung block is excised from the thorax. These inflated lungs are then placed in cold Perfadex solution and transported on ice at 4-8^{o}C to the recipient centre(63) (64).

Presently there is no evaluation of the lungs during the transport period and there is no opportunity to implement ongoing reparative strategies prior to their reimplantation in the recipient. The greatest obstacles in current donor preservation are the inability to continually repair and provide nutritive supplement to the lungs, and the inability to ventilate the donor lungs which, allows for ongoing delivery of oxygen and substrate,

during transport. As such, the current procurement strategy of cold static preservation is limited to a maximum 10-hour window. However, the current state of success using these strategies for donor procurement have been excellent.

Lung transplantation survival and quality of life post-surgery continues to improve over the last two decades. This is thought to be primarily due to advances in immunosuppression, cytomegalovirus matching, and improvements in size matching.

1.5.4 Donor lung criteria and lack of donor lung utilization

International Society for Heart and Lung transplantation ISHL has defined a strict criteria for lung donor acceptance: from (65)

- Age <55 years
- ABO compatibility
- Clear chest radiograph
- PaO2 >300 on FiO2 = 1.0, PEEP 5 cm H2O
- Tobacco history <20 pack-years
- Absence of chest trauma
- No evidence of aspiration/sepsis
- No prior cardiopulmonary surgery
- Sputum gram stain—absence of organisms
- Absence of purulent secretions at bronchoscopy

The published data on donor lung utilization is as low as <20% of potential donors (66). The causes for rejecting organs includes: unacceptable oxygen challenge, abnormal chest x-ray/CT/MRI results, bronchoscopy findings, and donation after cardiac death (DCD). All of these criteria for donor refusal may be repaired on ex-vivo assessment(67). Ex-vivo assessment will allow for protective ventilation strategies, and serial bronchoscopy

that will aid in lung recruitment and improve oxygenation. The greatest area for improvement in donor utilization is the ability to assess lungs outside of the body. This permit both repair of procured marginal lungs and long-distance transport without incremental damage to the lungs by ischemia.

1.5.5 The Evolving Donor Pool of Donation after Cardiac Death (DCD):

The main donor population pool consists mainly of those with brain death criteria that have not yet undergone cardiac arrest(68). In the last years, there has been an attempt to increase the utilization of donors that are pronounced dead due to cardiac arrest(69)(70)(71). As the donor pool has expanded in lung transplantation to use these donors after cardiac death, this provides new challenges for objectively evaluating these lungs(72)(73). In the setting of DCD donors, the procurement of organs can only begin after the heart has stopped, imposing a period of controlled warm ischemia on the lungs prior to surgical dissection and infusion of cold Perfadex solution through the main pulmonary artery. Lung compliance and lung vascular dynamics cannot be monitored, nor can their ability to oxygenate and effectively gas exchange in the DCD donor once the heart has stopped. Hence, experienced centres started this technique by assuming that prior to cardiac death that the lungs did not suffer significant injury from the warm ischemic time. At this point, they quickly procured the organs in a standard fashion 5 minutes after cardiac death was declared. The inflicted time of warm ischemia is known to be especially detrimental to lung function. That is, organ metabolism is not decreased by the cooling process that occurs exactly at the time of ischemia in the standard brain

death donors. This is why in the current modality of organ procurement organs are cooled during transportation to minimize metabolism and allow for organs to maintain viability for several hours. In the warm ischemic period viability of organs are drastically reduced to only a few hours. Lungs potentially can tolerate a warm ischemic insult of 60-90 minutes. In a routine DCD donor procurement, the warm ischemic time is usually 30-40 minutes. The ex-vivo lung assessment technology is well suited for this evolving DCD donor pool. The portable ex-vivo technology provides the advantage of allowing for the period evaluation of DCD donor lungs that have undergone a period of warm ischemic time. This ex-vivo technology will give clinicians an opportunity to objectively evaluate the donor lungs for lung function parameters, including saturations from the pulmonary venous blood, airway compliance, and pulmonary vascular resistance. This objective evaluation of donor lungs outside the body will be unique to ex-vivo platforms that permits for assessment of donor organs before committing to implantation from the DCD donor pool. This will also increase the potential number of donors of thoracic organs for transplantation in a safe and systematic fashion.

1.5.6 Advances in Lung Donor Procurement:

Stig Steen and colleagues, from Sweden, have pioneered the utilization of ex-vivo normothermic lung perfusion (EVLP) since 2001(68). The need to create this technology was created to aid the start of DCD donor lung transplantation nonetheless this technology can be extended to all donor lungs for objective assessment ex-vivo(70).

Furthermore, the original ex-vivo perfusion apparatus was developed to assess donor lungs at the recipient site, given that the technology was not portable. The first EVLP machines primarily used components from a cardiopulmonary bypass machine and an ICU ventilator (**Figure 2**). All of these non-portable devices where developed only to control ex-vivo perfusion of the lungs at the recipient site. Clearly, since the original utilization of the ex-vivo technology, rigorous methodological evaluation has occurred and improved the efficacy of the device. The original EVLP was only stable for two hours(74)(71), while today EVLP has been shown to be stable for up to 12 hours. The majority of this advancement has been performed by careful experiments with protective strategies by Shaf Keshavjee and the thoracic transplant program at Toronto General Hospital (74). The efficacy of prolonged EVLP has been shown to be better when compared cold static perfusion versus ex-vivo lung perfusion in a pig model of lung transplantation (75). Both study arms underwent cold static preservation after being procured from donor pigs for 12 hours. The ex-vivo arm then underwent ex-vivo lung perfusion for 12 hours while the cold static perfusion arm continued cold static perfusion for another 12 hours. At the end of 24 hours, the two lung groups underwent transplantation, 4 hours of reperfusion and then evaluation with peak airway pressures 4 hours post transplantation, lung oxygenation 4 hours post transplantation and at recipient sacrifice 4 hours later. They demonstrated the potential for superiority of ex-vivo lung perfusion in this model. In 2009, the Toronto group improved lung function by EVLP therapy, showing that adenoviral gene transfer on Interleukin(IL)-10 by bronchoscopic delivery reduced inflammation in the donor lungs(76). The ex-vivo adenoviral delivery of

gene therapy was associated with improved lung function of the transplanted lungs after 4 hours of reperfusion.

In 2011, the same group performed the first clinical trial using normothermic ex-vivo lung perfusion in human transplantation (77). Ex-vivo lung perfusion for 4 hours in 'high-risk' donors that were deemed potentially not suitable for transplantation was performed. They completed this on a total of 23 donors with 116 donors in the control arm. Of the 23 donors that received lungs that underwent ex-vivo lung perfusion due to the 'high-risk' nature of the donor, 20 were used for clinical transplantation. Of note, from the 20 lungs that were implanted after EVLP, 9 came from the DCD donor pool. This study concluded that ex-vivo lung perfusion was safe in a clinical setting. Two important conclusions from this work are firstly, it showed that marginal donors with lower oxygenation challenge tests can be placed on the EVLP system and undergo perfusion for 4 hours safely. Lungs shown to be stable with acceptable gas exchange and dynamic airway compliance were transplanted with acceptable clinical outcomes. Secondly, it showed that the DCD donor could be evaluated on this device for objectively for 4 hours to evaluate the quality of donor lungs prior to transplantation. This significant advance in donor procurement for lung transplantation opens the opportunity for further improvement.

Although previous work by Kashevjee and colleagues from Toronto has highlighted areas of advancement, there are significant issues with the device developed and used in Toronto. There device is non-portable, so lungs are exposed to long cold ischemic times

prior to starting ex-vivo perfusion. There device occupies a sterile operating room (OR) for several hours.

There device needs OR personnel, including a scrub nurse, to set up a sterile table of instruments, a perfusionist and/or clinician to manage the device. There device is complex, with several components of a cardiopulmonary bypass machine and ICU ventilator, requiring a highly trained individual to work and troubleshoot all aspects of their EVLP system. Finally, the device uses an acellular perfusate, which makes functional assessment more speculative.

1.6 Most recent advances in Ex- Vivo Lung Perfusion (EVLP) Apparatus

1.6.1 TransMedics Lung Organ Care System (OCS):

The TransMedics Lung OCS system was created to advance the current ex-vivo technology with the goal of providing a portable single unit of physiologic support. This drastically reduces the ischemic time to the lungs by eliminating 'travel' time. That is, the donor lungs are only ischemic for the time that it takes to cross-clamp the aorta, flush the lungs with Perfadex solution in the chest, explant the lungs from the thorax and place them on the portable ex-vivo device. This brings the ischemic time of several hours down to less than one hour with the portable system. As a result, immediate and sustained recruitment and resuscitation of the lungs ensues. It also provides continuous monitoring, assessment, and treatment, from the time the lungs are procured to the time they are ready

for reimplantation. Another advantage of the TransMedics Lung OCS system is the efficiency in utilization of resources, as it does not require another sterile surgical room and scrub nurse. It also obviates the need for a perfusionist as the device is fully automated with very simple instrumentation. Further to this, there is no need for suturing to the LA cuff of the donor because the cannulas are tied on to the pulmonary artery and trachea. The current TransMedics device does not change the conventional donor organ retrieval team. The same surgeon procuring the donor organs can place the lungs on the TranMedics Lung OCS. The portable monitor will allow the procuring surgeon to continuously assess the lungs during transport to the recipient centre. These features are all advantages needed at the University of Alberta, especially in view of the unique geographical isolation. Data acquisition is also built into the device. The device has two functional modalities during ex-vivo perfusion. Firstly, there is a preservation mode where lungs are continually perfused with a blood perfusate that is exposed to a membrane oxygenator at room air (fraction of oxygen = 21%), while also being ventilated with oxygen at room. Secondly, there is a monitoring and assessment mode where deoxygenated gas is filtered through the blood perfusate. This provides a functional assessment of the lungs through its ability to oxygenate the deoxygenated perfusate and therefore mimic physiological conditions of systemic venous blood circulated through the pulmonary artery. The perfusate is also novel, in that it is a more physiologic solution that includes blood perfused continuously through the lungs. The perfusate consists of a buffered solution with three units of packed red blood cells and additives.

The instrumentation of the Lung OCS system is relatively simple with cannula simply tied to the pulmonary artery a zip tie connection of the trachea to the endotracheal tube. The initial lung stabilization and preservation strategy is also well described, temperature is increased from 32^{o}C to 37^{o}C with tidal volumes of 6 ml/kg of donor weight and a positive end expiratory pressure of 5 cm of water with the respiratory rate of 10 breaths per minute. The portable monitor provides real time data collection of the following parameters; pulmonary blood flow in litres per minute, pulmonary artery pressures, pulmonary vascular resistance, the saturation of oxygen in the pulmonary venous blood, the saturation of oxygen in the pulmonary arterial blood, the hematocrit, the temperature of perfusate, the respiratory rate, airway pressures, and tidal volumes. The device maintains organ sterility and also allows for bronchoscopy and intervention during resuscitation of the organs. The device also has several built in safety checks to determine adequate flow and ventilation to the lungs. Should these parameters be abnormal, the device shows alerts on the monitor and troubleshooting options are available. These include increasing/decreasing flow of perfusate, increasing/decreasing ventilation rates and tidal volumes. It also allows for hand held recruitment manoeuvers with an external bagger. If there is device failure, the system will flush the lungs with cold Perfadex solution and the donor surgeon can subsequently store the lungs in cold static preservation for the rest of the transport period. All of these advantages make this system ideal for use in most of institutions.

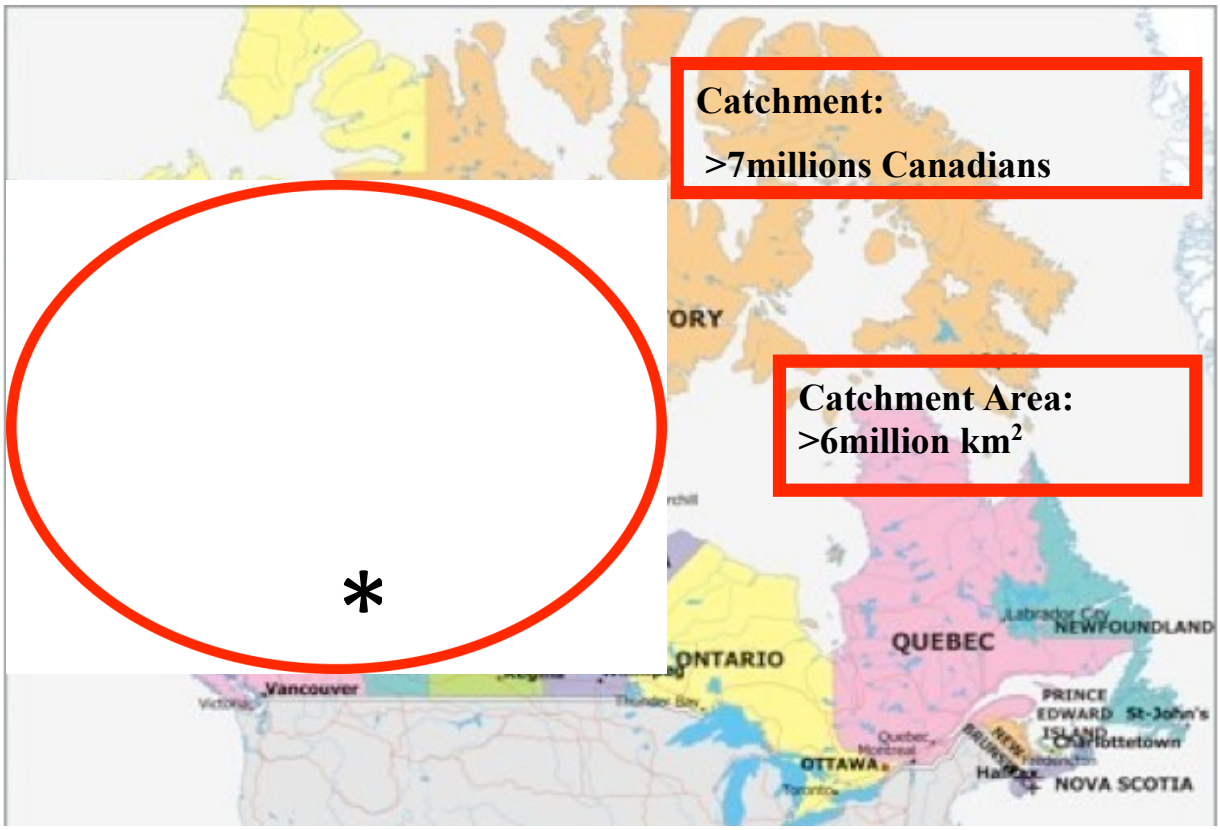
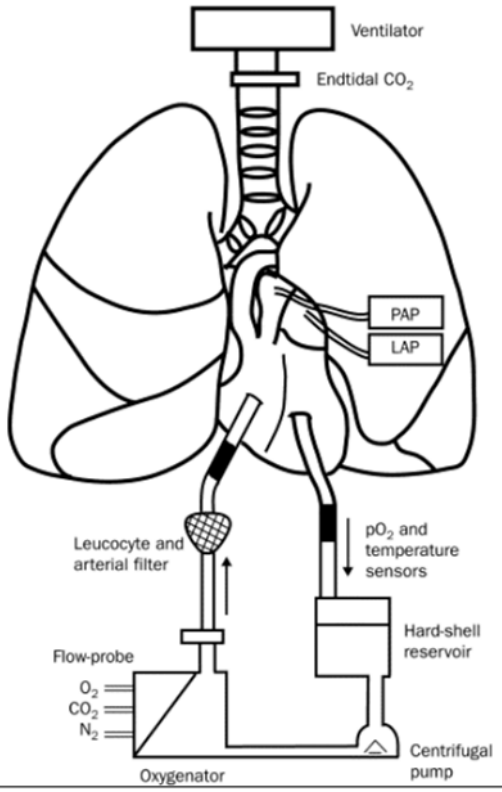


Figure 1 Geographic isolation of the University of Alberta

University of Alberta/Mazankowski Alberta Heart Institute performs the majority of thoracic transplantation for several provinces in Canada. The U of A represents the largest geographic catchment of any thoracic transplant program in North America and Europe. This leads to unique challenges with distance of potential donors and timing of bringing distant recipients to the center for transplantation.



•Schematic drawing of lung assessment ex-vivo as first performed in Sweden by Stig Steen and colleagues to help objectively evaluate non-heart-beating donors.
•The device is non-portable and requires a sterile OR room for it to be used
•The device incorporates components found in a cardiopulmonary bypass machine.

Steen et al. Transplantation of lungs from a non-heart-beating donor. *Lancet*. 2001; 357:825-9

Figure 2 Original Ex-vivo lung apparatus (68)

2 Chapter 2:

Hypoxic Pulmonary Vasoconstriction is a more accurate parameter than P/F ratio to measure lung function on Ex-Vivo Lung Perfusion

Hypoxic Pulmonary Vasoconstriction is a more accurate parameter than P/F ratio to measure lung function on Ex-Vivo Lung Perfusion

Abstract

Background: Rates of thoracic donor organ utilization remains very low at 15-25% internationally. Development of normothermic Ex-Vivo Lung Perfusion (EVLP) has shown to increase rates of donor lung utilization. Adoption of EVLP to routine clinical practice will require more sensitive physiological parameters of lung function on EVLP. Currently, P/F ratio has been used as the most reliable parameter of lung function on EVLP. Our data suggests a change in the lungs response to hypoxic pulmonary vasoconstriction (HPV) is a more sensitive parameter to detect early lung dysfunction on EVLP.

Methods: Our novel EVLP platform as an apparatus for large animal model and human pre-clinical/clinical investigation, has two centrifugal flow pumps (instead of one) and it is equipped with a computer-controlled feedback system that provides real-time control of PA and LA pressures/flows as well as monitoring/recording the ventilator parameters (such as peak/plateau pressures, PEEP) and lung perfusion data (i.e. PVR) with 10sec intervals. We challenged the lungs with hypoxia serially during the EVLP run by ventilating the lungs with 100% N2 to observe the response of hypoxic pulmonary vasoconstriction (HPV).

Results: We performed EVLP on 10 domestic pigs (35-45kg) for 12 hours each. We found our device provided excellent lung function while on EVLP up to 12 hours, with the P/F ratio remaining >400 mmHg throughout. EVLP was maintained at 75% cardiac output (2.2-2.4 L/min), where PA pressure and LA pressure are controlled constant by the device software at 8 mmHg and 2 mmHg, respectively. Lung compliance remained stable up to 12 hours without significant changes to peak/mean airway pressures, and PVR. When lungs were challenged with hypoxia, there was a serial blunting in HPV response over the course of 12 hours with a decreased rise in PVR when challenged with hypoxia ($p<0.05$).

Conclusions: As experience is gained with EVLP, appreciation of the most sensitive parameters for donor lung function is critical to allow for extended EVLP runs, and to assess therapeutic interventions. The conventional use of P/F ratios may not reflect sub-clinical lung dysfunction, while response to HPV may be a more sensitive physiologic measurement of lung function.

2.1 Introduction

The University of Alberta has one of the largest lung transplant centers in Canada. Alberta lung transplant program covers a catchment population of more than 7 million Canadians in the provinces of Alberta, Northern British Columbia and Saskatchewan (**Figure 1**). Among all the provinces of the country, Alberta has the lowest organ donation rate which affect the chances of patients on the lung transplant waitlist to receive a transplant. Another unique challenge to the thoracic transplant center at the University of Alberta is being the most geographically isolated program in North America and Europe which makes the procurement of donor transplant organ challenging(1).

Lung transplantation is the only lifesaving treatment for patients with end stage lung disease as it has been shown to increase the survival rate for those patients following lung transplantation(2). Additionally, more than one study on patient's health status, functional status and adjustment after lung transplantation have demonstrated a significant improvement in quality of life (QOL) outcomes following lung transplantation comparing to the QOL before receiving a lung transplant(3)(4)(5).

So experiments taking place in our laboratory have been focused on helping one of the most vulnerable patient population in medicine, patients with chronic end stage lung

disease (ESLD) (6). Our overall objective is to increase the pool of donor thoracic organ and increase the rate of organ utilization. The greatest area for improvement in donor utilization is the ability to assess lungs outside of the body by using normothermic ex-vivo lung perfusion (EVLP). EVLP is a technology that gives us the opportunity to assess, evaluate and repair donor lungs outside of the body. This permits both reduce ischemia time caused by long distance of available donor lung and repair marginal donors before transplant. This technology will allow for objective evaluation of DCD donor lungs after exposure to a period of warm ischemia which will further increase the donor population and donor utilization of lungs. This will lead to a positive impact for patients on lung transplantation wait list.

Despite all improvements in organ donation, rates of thoracic donor organ utilization remains very low at 15-25% internationally. Development of normothermic Ex-Vivo Lung Perfusion (EVLP) has shown to increase rates of donor lung utilization. Our lab is focusing on improving the EVLP platform to provide better control of pressure and flow.

Adoption of EVLP to routine clinical practice will require more sensitive physiological parameters of lung function on EVLP. Currently, P/F ratio and physiological parameters (Lung compliance, airway pressure and resistance) have been used as the most reliable parameter for monitoring lung function on EVLP(83). Despite that, using EVLP in clinical practice to assess lungs before transplantation requires an additional investigations.

2.2 Hypoxic pulmonary vasoconstriction (HPV)

The normal response of the intrapulmonary arteries to hypoxia is constriction unlike the response of systemic arteries. Hypoxic pulmonary vasoconstriction (HPV) is a very mandatory physiological response to alveolar hypoxia to shift blood to a better oxygenated area in the lungs to optimize ventilation/perfusion (V/Q) ratio, thereby improving oxygenation in case of hypoxia(84). That's believed to be done by the mitochondria which in a response to acute hypoxia it changes reactive oxygen species in pulmonary smooth muscle cells, which leads to depolarizing pulmonary artery smooth muscle cells (PASMC) by inhibiting potassium channels (85). After that Calcium channels activates and increases calcium which will lead to a vasoconstriction (86).

Intrapulmonary arteries in response to a generalized hypoxia, hypoxic pulmonary vasoconstriction response would raise Pulmonary Vascular Resistance (PVR), which can be useful in assessing pulmonary vascular endothelial dysfunction. In a healthy lungs the change in PVR will be high while in a diseased lungs with pulmonary vascular endothelial dysfunction there will be no change in PVR (blunting effect) (87).

So, we hypothesize that hypoxic pulmonary vasoconstriction (HPV) during Ex-Vivo Lung Perfusion is a more accurate parameter than P/F ratio to measure lung function to determine donor lung quality prior to transplantation and is a more sensitive parameter to detect lung dysfunction during EVLP.

2.3 Objectives

To prove that our novel EVLP circuit with two centrifugal pumps allows for stable normothermic EVLP for over 10 hours, as the P/F ratio remains acceptable throughout. We sought to compare the P/F ratio and hypoxic pulmonary vasoconstriction as metrics of donor lung function during ex vivo heart perfusion.

2.4 Methods

2.4.1 Edmonton EVLP platform: A novel platform for Ex-vivo Lung Perfusion

Our novel EVLP platform as an apparatus for large animal model and human pre-clinical/clinical investigation, has two centrifugal pumps (instead of one) and it is equipped with a computer-controlled feedback system that provides real-time control of PA and LA pressures/flows as well as monitoring/recording the ventilator parameters (such as peak/plateau pressures, PEEP) and lung perfusion data (i.e. PVR) with 10sec intervals **(Figure 3).** EVLP platform has an oxygenator, a heat exchanger, a reservoir, leukocyte filter, ventilator system and a hypoxic gas mixture (86% N_2, 8% CO_2, 6% O_2). We challenged the lungs with hypoxia serially during the EVLP run by ventilating the lungs with 100% N2 to observe the response of hypoxic pulmonary vasoconstriction (HPV). Hypoxic ventilation was accomplished by titrating the FiO2 from 21% to 0% using nitrogen.

2.4.2 Animal Model

Female Yorkshire pigs with a target weight of 35 kg to 45 kg were used. Received research ethical approval from the Animal Care and Use Committee for Health Science in University of Alberta.

2.4.3 Steen solution (Perfusate)

Stig Steen and colleagues from Toronto were able to develop an acellular buffered solution to be used as a perfusate in Ex- vivo Lung perfusion circuit(68). Steen solution has an optimal colloid osmotic pressure and this will help in maintaining a stable lung function during EVLP without edema formation. The solution is ideal for the Ex-vivo assessment of marginal lung function and therefore, expanding donor lung pool.

Steen Solution compose of:

- Calcium chloride
- Magnesium chloride
- Sodium chloride
- Potassium chloride
- Sodium dihydrogen phosphate Glucose
- Sodium bicarbonate Water
- Dextran 40, coats and protects endothelium from thrombogenesis
- Human serum albumin, provide a normal physiological oncotic pressure which decrease edema formation

Steen's group were able to perfuse and evaluate animal model lungs for one hour without pulmonary edema formation followed by a successful transplantation by using a mix of red blood cells and Steen solution as a perfusate (68).

2.4.4 Lung procurement

Yorkshire pigs (35-45kg) will be induced with an intramuscular injection of Atropin (0.5 mg/kg), Ketamine (20 mg/kg), anesthetized with inhaled isoflurane (5%) and maintained with propofol (5–8 mg/kg/h I.V). Orotracheal intubation will be established for institution of mechanical ventilation and maintenance of general anesthesia using 5% inhalational isoflurane. Pigs will be mechanically ventilated to maintain a pH of 7.35 to 7.45, PO_2 >200 mmHg, and PCO_2 of 35-45 mmHg.(88)

A median sternotomy will be performed by cautery and sternal saw. Remove thymus gland and systemic heparinization will be established by delivery of 40,000 units of heparin through superior vena cava. The pericardium will be opened with sharp dissection. We cannulate the pulmonary artery and flush through it with a delivery of (3.0 L) of Perfadex antegrade. After flushing the lungs with prefadex donor exsanguination stars with a cut at the superior vena cava and suction of blood into gas jar (for autologous whole blood experiments) or into a Brat2 cell saver, which separates donor blood into packed red blood cells and plasma components (for autologous packed RBC experiments). After euthanization by donor heart excision, Pneumonectomy will start by freeing the lungs from ligaments around which attached to the diaphragm and free the trachea from tissue attached to the esophagus, then pneumonectomy will end by cutting pulmonary artery, left atrium and clamped trachea after ventilation. The excised lungs will be weighed and placed in a container filled with ice-cold saline solution. The lungs

will be taken up to our laboratory where all further experiments will be conducted on the ex-vivo lung perfusion apparatus.

2.4.5 Ex-vivo Lung Perfusion

As explained by Cypel et al (74). After lungs are procured, we cannulate the pulmonary artery and left atrium. Then we intubate the trachea with endotracheal tube. EVLP circuit was primed before lung procurement with (1L) of Steen Solution, Methylprednisolone (500 mg), Piperacillin/tazobactam (3.375 g), Insulin (20 units) and we add 0.5L of autologous whole blood. We start perfusion when lungs were placed in ex-vivo dome and connected to EVLP circuit with the following parameters: pulmonary artery flow (PA flow) was maintained at (2.2-2.4 L/MIN), PA and LA pressures were controlled at 8 mmHg and 2 mmHg, respectively. During the first hour of EVLP run we maintained the flow around 40% of cardiac out and we gradually increase the temperature to reach 37°C. Ventilation started when temperature reached > 32°C and ventilator sittings were as follow: Tidal volume (V_T) = 6ml/kg, Respiratory rate = 8 breaths / minute, Fraction of inspired Oxygen (FIO_2) = 21% and PEEP = 5cm H_2O. We deliver 1L of hypoxic gas mixture or Sweep gas (86% N_2, 8% CO_2 and 6% O_2) to the oxygenator to deoxygenate the pulmonary artery line which is entering the lung and this should result in PO_2 of around 60 mm Hg. Our computer controlled feedback system records the ventilator parameters (such as peak/plateau pressures, PEEP) and lung perfusion data (i.e. PVR) with 10sec intervals. We have collected a perfustae samples every two hours during the

EVLP run for ELISA (TNF-α and IL6). Finally, Hypoxic ventilation was accomplished by titrating the FiO_2 from 21% to 0% for 4 minutes using nitrogen. (83)

2.4.6 Statistics

All results are expressed as mean ± SEM. 1–way (ANOVA) was utilized and $p<0.05$ was considered statistically significant.

2.5 Results

2.5.1 Lung oxygenation (P/F ratio) during ex vivo lung perfusion

We performed EVLP on 10 domestic pigs (35-45kg) for 12 hours each. We found our device provided excellent lung function while on EVLP up to 12 hours, with a stability of physiological parameters. The peak airway pressure (PAWP) remained stable from 18 cm H_2O to 22 cm H_2O **(Figure 4).** The compliance was from 16 ml/cm H_2O TO 26 ml/cm H_2O **(Figure 5).** Arterial partial pressure of oxygen (PaO_2) was recorded through the experiment and was physiologically normal from 70 mmHg to 100 mmHg while when the lungs were challenged with hypoxia by infusing 100% medical N_2 , (PaO_2) was reduced and ranged from 27 mmHg to 41 mmHg **(Figure 6).** The P/F ratio remained >400 mmHg throughout **(Figure 7)**. EVLP was maintained at 75% cardiac output (2.2-2.4 L/min), where PA pressure and LA pressure are controlled constant by the device software at 8 mmHg and 2 mmHg, respectively. So, our novel EVLP circuit with two centrifugal pumps allows for stable normothermic EVLP for over 10 hours, as the P/F ratio remains acceptable throughout.

2.5.2 TNF-alpha concentration during ex vivo lung perfusion

During EVLP run, we collected perfusate samples in 2 ml Eppendorf tubes every 2 hours of perfusion run to analyze it by enzyme-linked immunosorbent assay (ELISA) for proinflammatory cytokine: Tumor necrosis factor alpha (TNF-α). Results showed that there is evidence of accumulation of proinflammatory cytokine (TNF-α) after 4 hours of EVLP (**Figure 8**), despite adequate oxygenation measured by P/F ratios. This result suggests there is a sub clinical lung dysfunction.

2.5.3 Interleukin 6 (IL6) concentration during ex vivo lung perfusion

Analyzing perfusate samples by ELISA for other proinflamatory cytockines IL6 showed that the is accumulation of IL6 in the first 4 hours of perfusion run (**Figure 9**) while oxygenation of the lung measured by P/F ration is not affected, again it is an indication of subclinical lung injury.

2.5.4 Pulmonary vascular resistance (PVR) reaction in response to hypoxic ventilation

When we challenged the lungs on EVLP with serial hypoxic ventilation (accomplished by titrating the FiO2 from 21% to 0% using nitrogen for 4 minutes) every 2 hours through perfusion run we interestingly noticed that there is a decline in HPV responsiveness (**Figure 10**) that correlates to the increase in inflammation observed over time despite adequate oxygenation measured by P/F ratios.

2.6 Conclusion

Our novel EVLP circuit with two centrifugal pumps allows for stable normothermic EVLP for over 10 hours, as the P/F ratio remains acceptable throughout.

There is evidence of accumulation of proinflammatory cytokines after 4 hours of EVLP (TNF-α and IL6), despite adequate oxygenation measured by P/F ratios. These result suggests there is a sub clinical lung dysfunction. Interestingly, there is a decline in HPV responsiveness that correlates to the increase in inflammation observed over time.

Finally, Hypoxic pulmonary vasoconstriction may be a more sensitive metric of donor lung dysfunction during ex vivo lung perfusion.

Ex vivo lung perfusion (EVLP) has been successfully used to expand the pool of organs available for lung transplantation. The capacity of the donor lung to oxygenate the perfusate solution can be estimated by measuring the P/F ratio. The P/F ratio is commonly used to select organs that are suitable for transplant and it is considered a gold standard parameter for evaluating in vivo donor lung; however, subclinical lung dysfunction may be present despite a normal P/F ratio during ex vivo lung cellular perfusion(74)(69) as showed by our data and other research groups. Toronto group conducted a study on physiological assessment of the ex vivo donor lung for transplantation(83). The aim of their study was to evaluate the parameters used to assess donor lungs on ex vivo sittings compared to what is used to assess lungs in vivo. They had an injured group (10 hours of brain death) and uninjured group (control lungs), they

measured PO_2, compliance and airway pressure during EVLP. They showed that PO_2 was normal in both groups during 12 hours of EVLP, while compliance significantly increased in the injured group after 1 hour of EVLP and airway pressure significantly increased in injured lungs after 3 hours of EVLP. Combining these results to what we have showed in our experiments we can say that PO_2 can be normal despite lung perfusion injury occurs during EVLP and depending on P/F ratio alone to assess donor lung in ex vivo sittings is not enough and might be misleading about the viability of donor lung for transplant. Additionally, Changes in compliance, airway pressures, accumulation of pro inflammatory cytokines (TNF-α and IL6) and a decline in HPV responsiveness during EVLP preceded any decrease in P/F ratio suggesting to be a more sensitive and earlier physiological parameters to assess and detect lung injury during ex vivo lung perfusion.

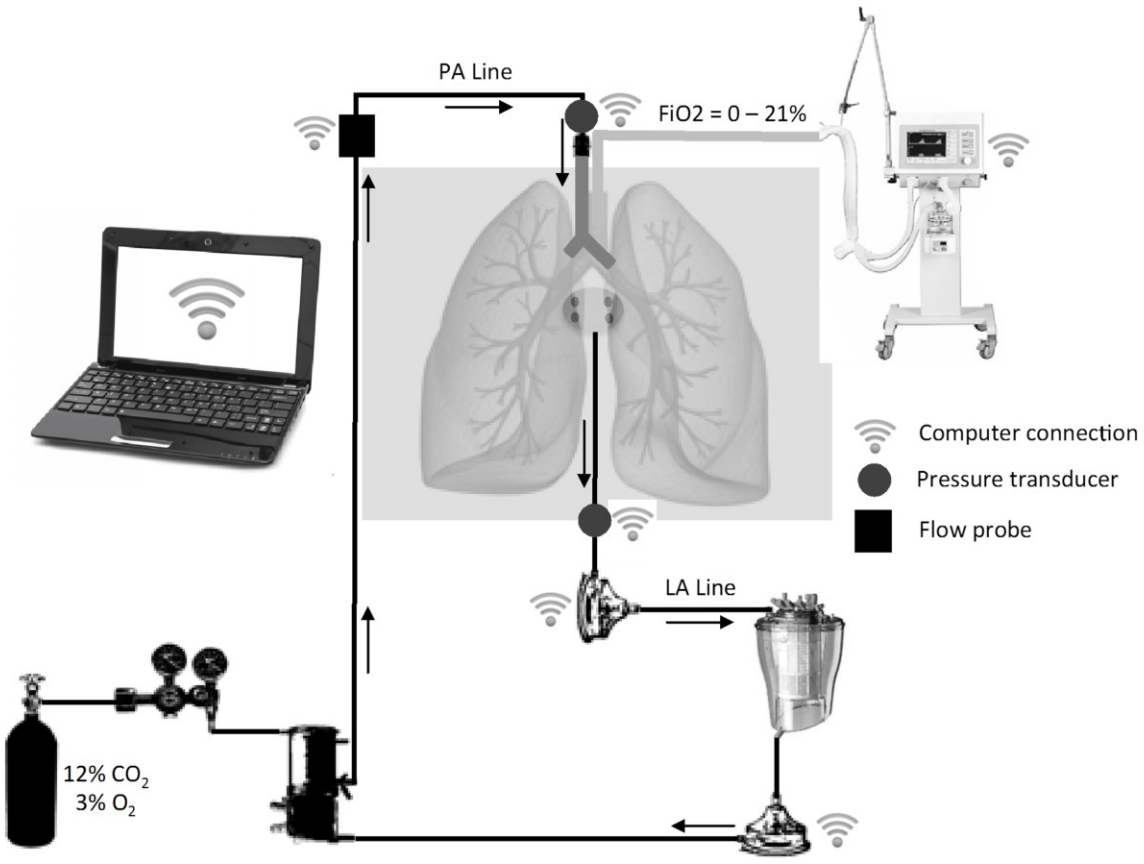


Figure 3 Edmonton EVLP platform: A novel platform for Ex-Vivo Lung Perfusion

Automated computer control of the LA line centrifugal pump and PA line centrifugal pump were used to maintain left atrial and pulmonary artery pressures, respectively.

Delivery of a hypoxic gas mix into the oxygenator was used to deoxygenate the perfusate solution delivered into the pulmonary artery.

Hypoxic ventilation was accomplished by titrating the FiO2 from 21% to 0% using nitrogen.

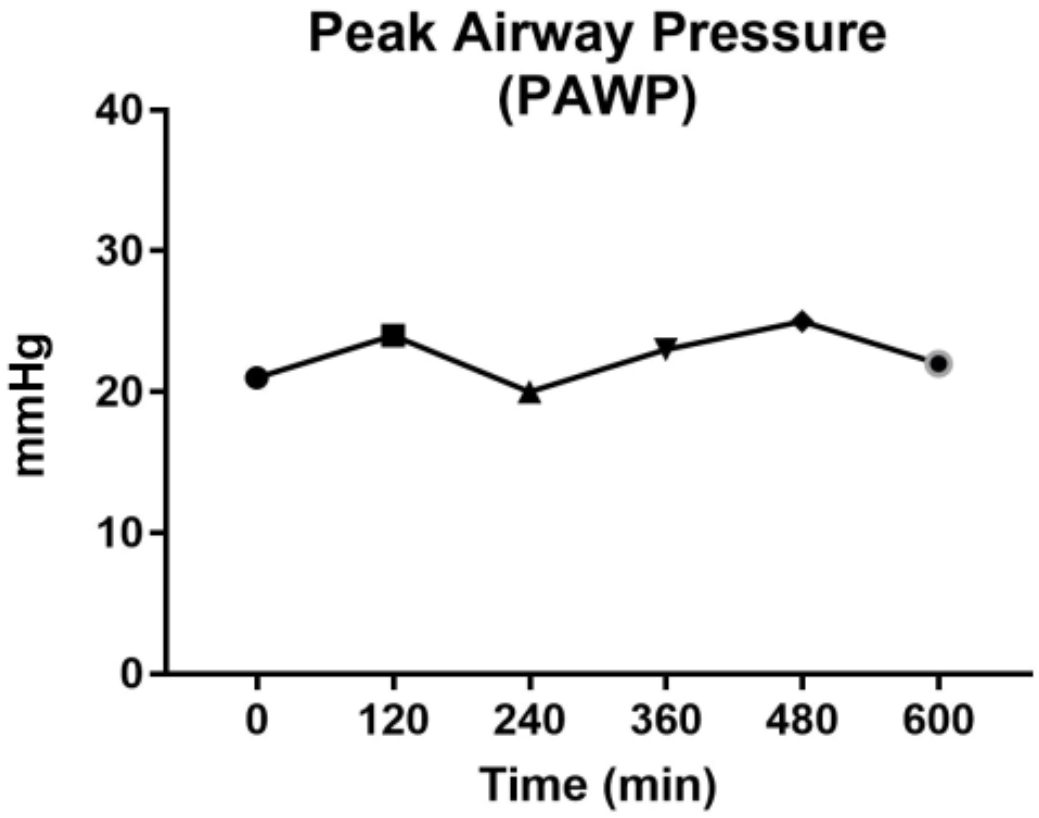


Figure 4 The peak airway pressure (PAWP)

The peak airway pressure (PAWP) remained stable from 18 cm H_2O to 22 cm H_2O ($p>0.05$).

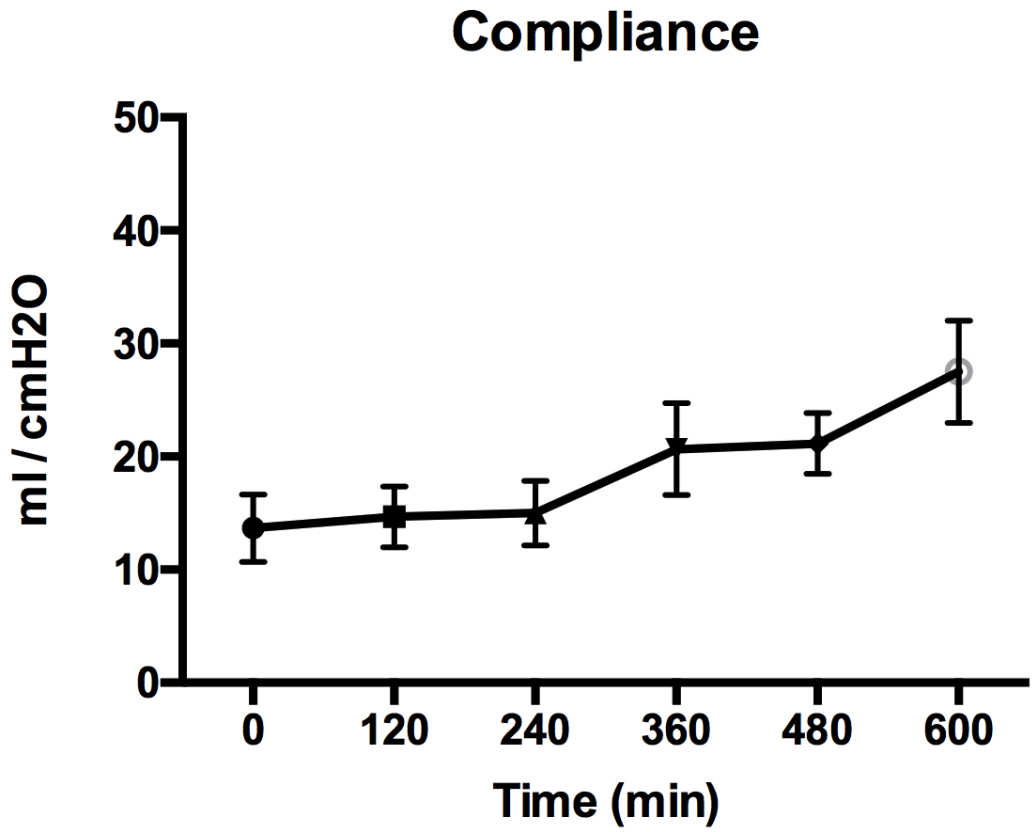


Figure 5 The compliance

The compliance remained in the normal range throughout the experiment ranging from 16 ml/cm H_2O TO 26 ml/cm H_2O. ($p>0.05$)

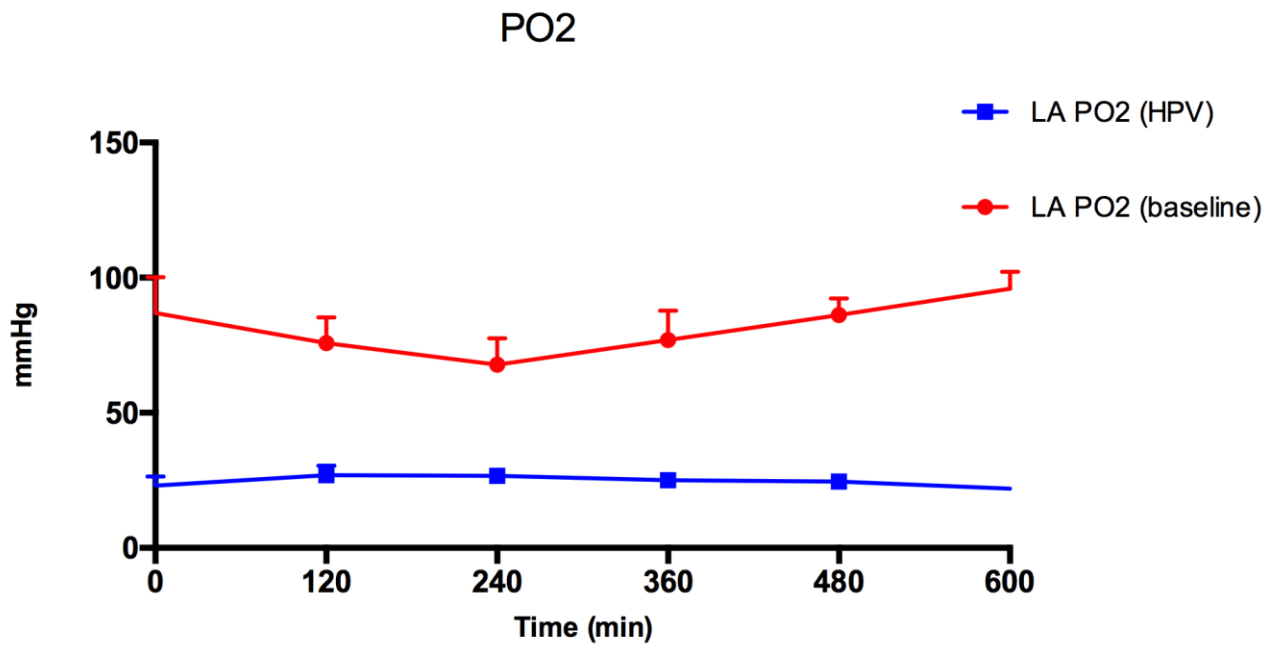


Figure 6 Arterial partial pressure of oxygen (PaO_2)

Arterial partial pressure of oxygen (PaO_2) was recorded through the experiment and was physiologically normal from 70 mmHg to 100 mmHg while when the lungs were challenged with hypoxia by infusing 100% medical N_2 , (PaO_2) was reduced and ranged from 27 mmHg to 41 mmHg.

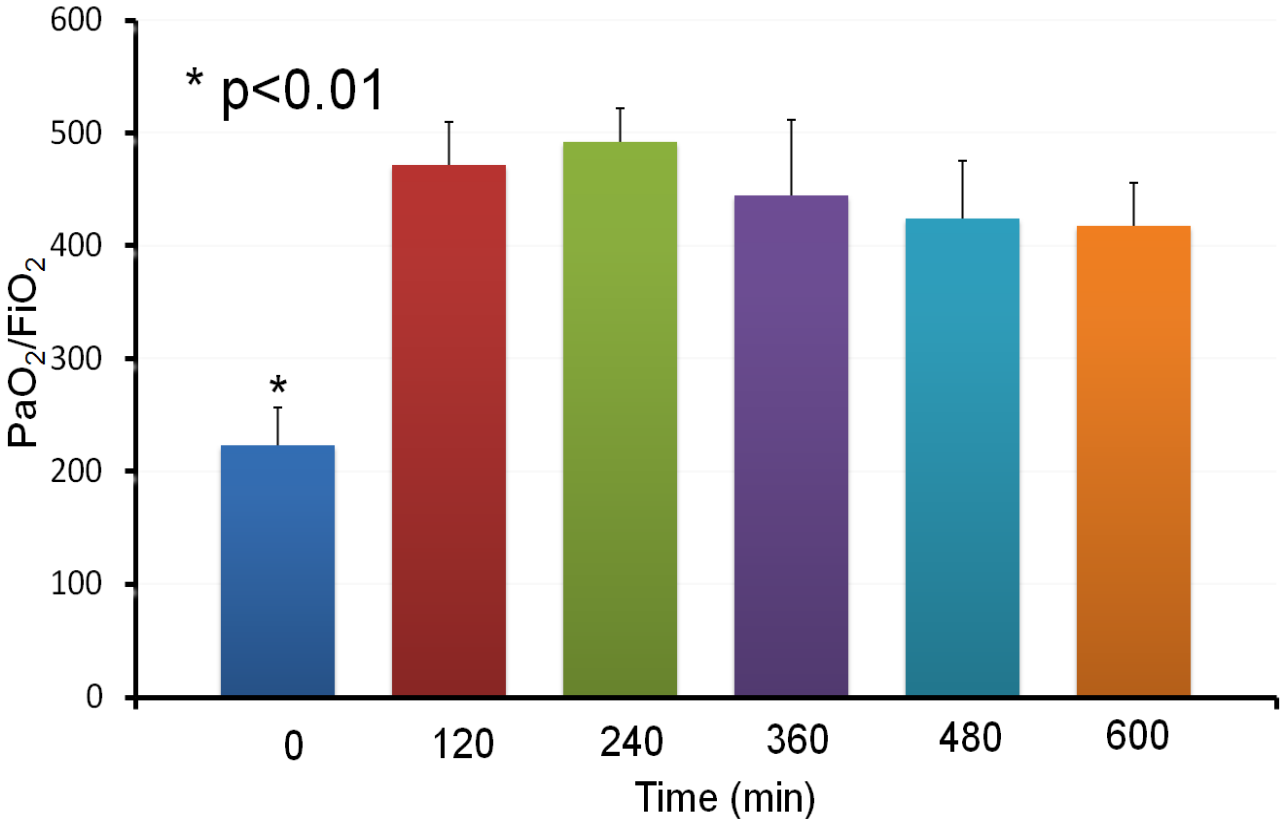


Figure 7 Lung oxygenation (P/F ratio) during ex vivo lung perfusion.

We performed EVLP on 10 domestic pigs (40-45kg) for 12 hours each. We found our device provided excellent lung function while on EVLP up to 12 hours, with the P/F ratio remaining >400 mmHg throughout. EVLP was maintained at 75% cardiac output (2.2-2.4 L/min), where PA pressure and LA pressure are controlled constant by the device software at 8 mmHg and 2 mmHg, respectively. Lung compliance remained stable up to 12 hours without significant changes to peak/mean airway pressures. So, our novel EVLP circuit with two centrifugal pumps allows for stable normothermic EVLP for over 10 hours, as the P/F ratio remains acceptable throughout.

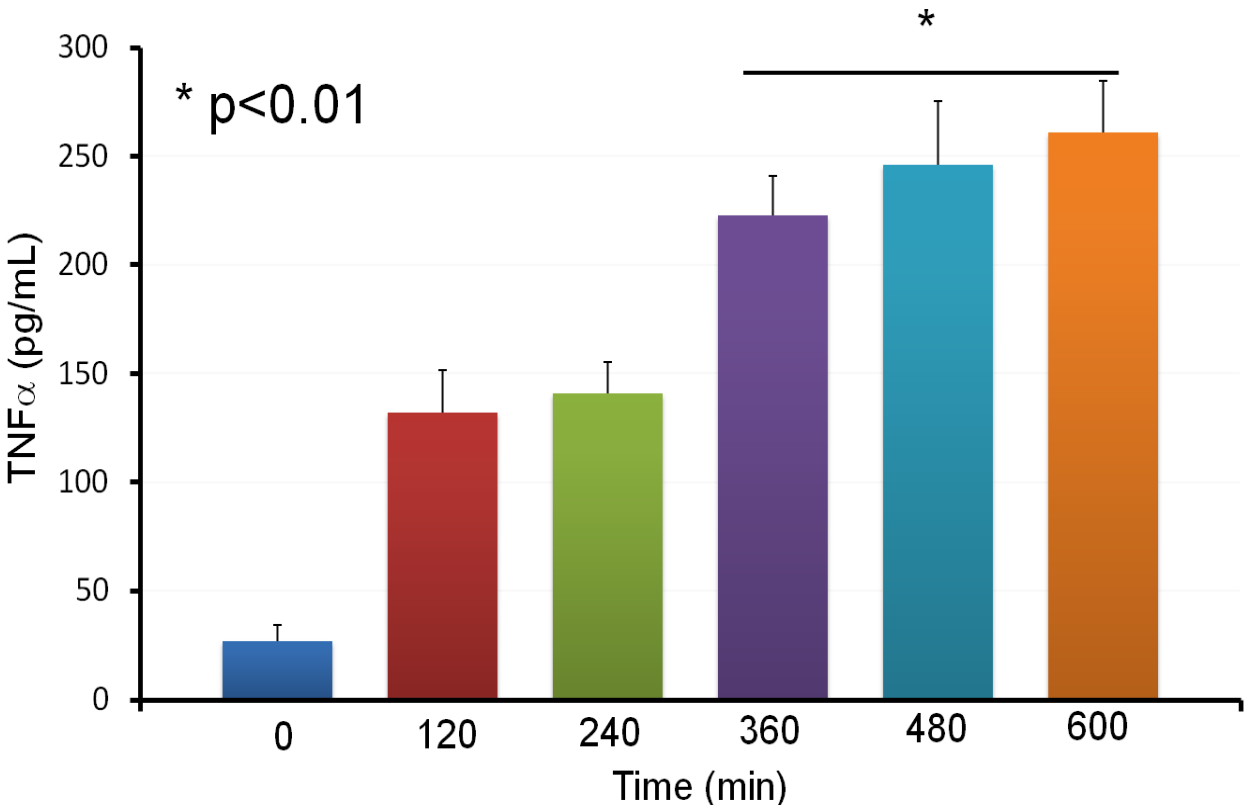


Figure 8 TNF-alpha concentration during ex vivo lung perfusion

Analyzing perfusate sample by (ELISA) for proinflammatory cytokine: Tumor necrosis factor alpha (TNF-α). Results in this figure showed that there is evidence of accumulation of proinflammatory cytokine (TNF-α) after 4 hours of EVLP, despite adequate oxygenation measured by P/F ratios. This result suggests there is a sub clinical lung dysfunction.

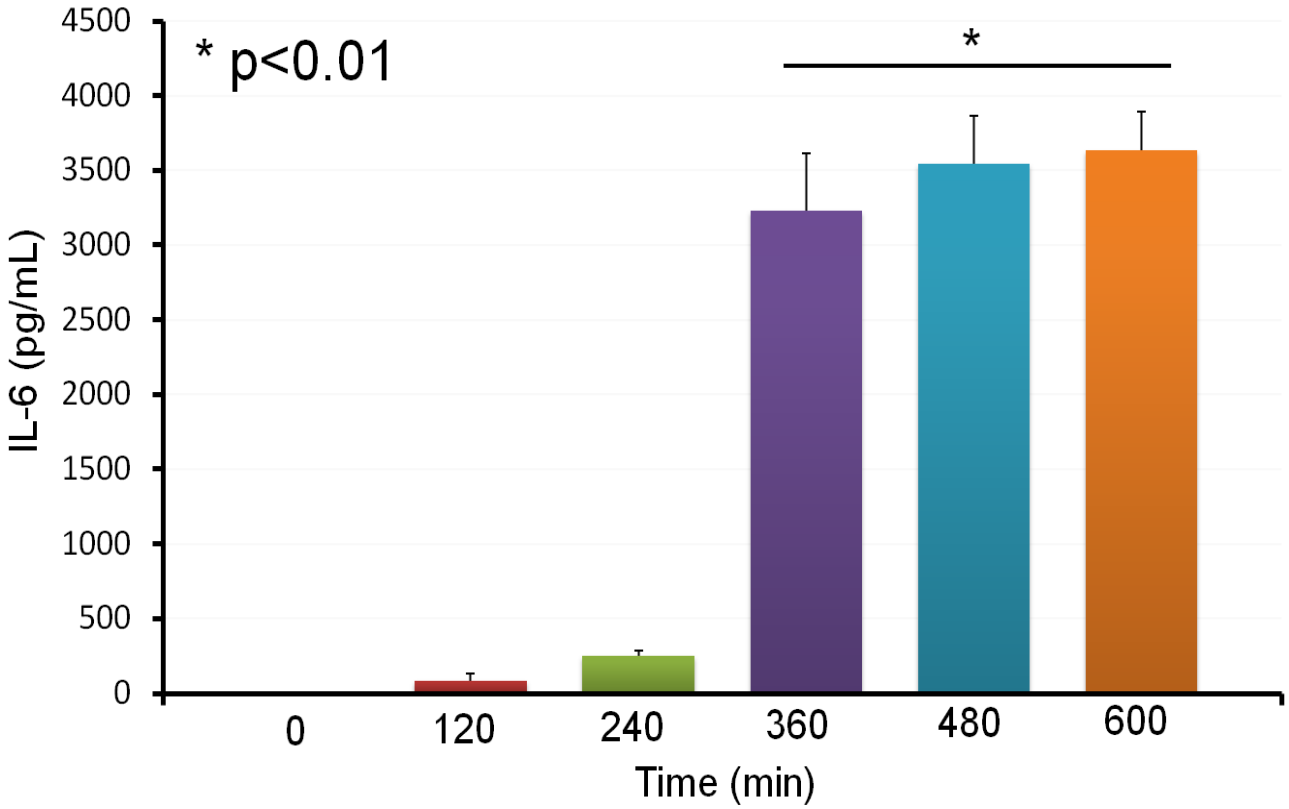


Figure 9 Interleukin 6 (IL6) concentration during ex vivo lung perfusion

Analyzing perfusate samples by ELISA for other proinflamatory cytockines IL6 showed that the is accumulation of IL6 in the first 4 hours of perfusion run while oxygenation of the lung measured by P/F ration is not affected, again it is an indication of subclinical lung injury.

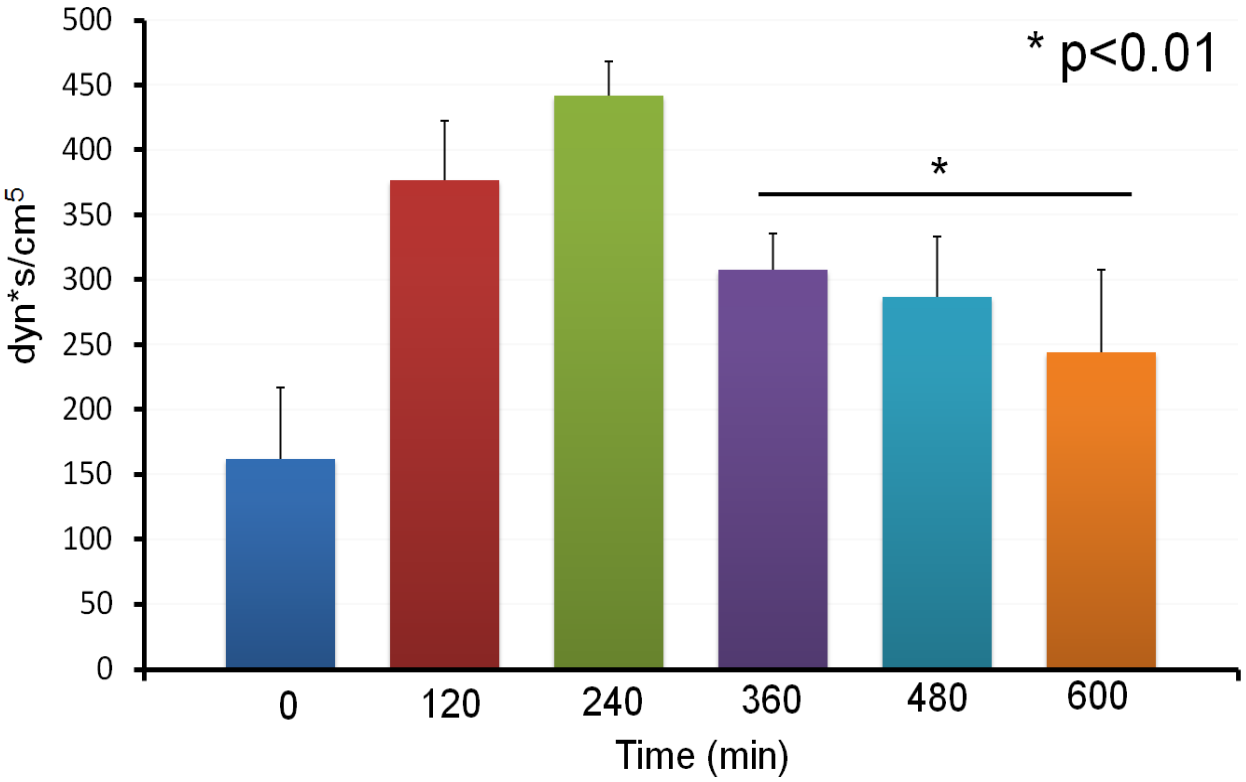


Figure 10 Pulmonary vascular resistance (PVR) reaction in response to hypoxic ventilation

When we challenged the lungs on EVLP with serial hypoxic ventilation (accomplished by titrating the FiO2 from 21% to 0% using nitrogen for 4 minutes) every 2 hours through perfusion run we interestingly noticed that there is a decline in HPV responsiveness that correlates to the increase in inflammation observed over time despite adequate oxygenation measured by P/F ratios.

3 Chapter 3:

Conclusion and Further directions

3.1 Conclusion and further directions

Lung transplantation is the only lifesaving treatment for patients with end stage lung disease as it has been shown to increase the survival rate for those patients following lung transplantation(2). The published data on donor lung utilization is as low as <20% of potential donors (66). The causes for rejecting organs includes: unacceptable oxygen challenge, abnormal chest x-ray/CT/MRI results, bronchoscopy findings, and donation after cardiac death (DCD). All of these criteria for donor refusal may be repaired on ex-vivo assessment.

Our overall objective is to increase the pool of donor thoracic organ and increase the rate of organ utilization by using normothermic ex-vivo lung perfusion (EVLP). This permit both repair of procured marginal lungs and long distance transport without incremental damage to the lungs by ischemia.

Adoption of EVLP to routine clinical practice will require more sensitive physiological parameters of lung function on EVLP. Currently, P/F ratio has been used as the most reliable parameter of lung function on EVLP. However, our data suggest there is evidence of accumulation of proinflammatory cytokines after 4 hours of EVLP (TNF-α and IL6), despite adequate oxygenation measured by P/F ratios. Furthermore, our data showed that change in the lungs response to hypoxic pulmonary vasoconstriction (HPV) is a more sensitive parameter to detect early lung dysfunction on EVLP.

Pulmonary artery vasoconstriction in response to hypoxia is a physiologic mechanism to optimize V/Q matching. Quantification of this hypoxic pulmonary vasoconstriction (HPV) response has been proposed as an alternative metric for assessing donor lungs during EVLP.

Further directions: Kakishita evaluated the change in proinflammatory cytokines of the perfusate during Ex-vivo Lung perfusion and investigated the effect of cytokine removal using an adsorbent membrane(89). Adsorbant membrane removal of proinflammatory cytokines was reported In Vitro and the rates were 99.9% for IL-8, 98.5% for IL-113, 82.9% for IL-6, and 31.2% for tumor necrosis factor (TNF-a)(90). Kakishita showed that tumor necrosis factor-a (TNF-α) and interleukin 8 levels were significantly lower in the adsorbant membrane group than in the control group during the EVLP period. I expect that by adding adsorbent membrane (**Figure 15**) to our Novel EVLP platform, proinflammatory cytokines concentration will be decreased.

Gene therapy by adenoviral vector delivery of transgenes (such as Adenosine monophosphate-activated protein kinase AMPK) is possible on the Lung OCS and is associated with decreased inflammation. Adenosine monophosphate-activated protein kinase (AMPK) is an important energy sensor that regulates functions in the cell (91)(92) such as energy utilization and energy hemostasis in the cell. It has been proved that AMPK has a role in cell response to stress and damage (93). This made researchers to design a couple of experiments on AMPK cell protection ability. They showed that AMPK can protect organs against ischemia-reperfusion and hemorregic shock injury by

decreasing inflammation (94)(95)(96). During reperfusion, AMPK modifies multiple cellular events such as fatty acid and glucose transport and utilization in the attempt to restore ATP levels back to their pre-ischemic levels. It is also an anti-inflammatory, decreasing levels of tumour necrosis factor-alpha (TNF-α) (97). As such by decreasing the inflammatory response in donor lungs, adenoviral transfer of AMPK may decrease the ischemia reperfusion injury and decrease the antigenic stimulation of the donor pig lung once transplanted into the recipient pigs.

A study by Adrian and colleagues reported increased level of (IL6) after 6 hours of Ex-vivo Lung perfusion(98), and Based on our data, we can tell that Lungs on Ex-vivo Perfusion will suffer from subclinical lung injury caused by accumulation of proinflammatory cytokines after 4 hours of EVLP (TNF-α and IL6).

Therefore, adding some anti-inflammatory drugs to the perfusate could be a further direction. Resveratrol has been proved to have an anti-inflammatory and antioxidant activities(99). An experiment by Wei pan showed that Resveratrol can protect against TNF-α induced injury in human umbilical endothelial cells(100). So, it is interesting to add Resveratrol to the perfusate just prior to the accumulation of proinflammatory cytokines (hour 4 of EVLP run based on our data), I hypothesis that there will be a decrease in cytokines concentration and therefore, decrease lung injury and improve function on EVLP. Improving perfusate compositions will have a great effect on the pool of donor lung utilization.

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