A Novel Experimental Porcine Model to Assess the Impact of Differential Pulmonary Blood Flow on Primary Graft Dysfunction After Unilateral Lung Transplantation

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Abstract

Background Primary graft dysfunction (PGD) remains a major obstacle after lung transplantation. We developed a novel approach to selectively assess reperfusion injury in transplanted lung and investigated the impact of pulmonary flow.

Materials Twelve porcine left lung transplants were divided in two groups (n = 6, in low (LF) and high flow (HF) group). Donor lungs were stored for 24 hours on ice, followed by left lung transplantation. Recipient animals were observed for 6h after reperfusion with partially clamping of right PA in HF group.

Results Survival at 6 hours was 100% in both groups. P_aO_2/F_iO_2 (P/F) ratio of the transplanted lung was lower (229.1 mmHg vs 308.9 mmHg, p=0.01), mPAP was higher (34.62 mmHg vs 29.88 p=0.01) in HF versus LF group. Wet-to-dry (W/D) ratios of right native and left transplanted lung were not different after 6 h of reperfusion.

Conclusions Lung transplantation in large animal settings is feasible. P/F ratio in allograft is dependent on PA flow. Our innovative approach to control blood flow to transplanted lung demonstrates that higher flows induce more PGD and allows to further study development and treatment of PGD. Our findings might have an important impact on sequential intra-operative approach during lung transplantation.

Introduction

Primary graft dysfunction (PGD) occurs within the first 72h after lung transplantation (LTx) and it is clinically reflected by impaired gas exchange, alveolar infiltrates on chest x-ray, and pulmonary edema representing acute allograft ischemia-reperfusion injury (IRI). [1] PGD remains one of the major obstacles following LTx and is the leading cause of early morbidity and mortality leading to prolonged intubation time and intensive care unit (ICU) stay. [2] The hallmark of PGD is the increased permeability of the alveolo-capillary membrane. The endothelium plays an important role in the maintenance of this membrane. Activation and injury can lead to increased permeability and accumulation of extravascular lung water into the interstitium. Also, the vascular endothelium represents the interface between blood and tissue for vascular permeability, inflammation and immune signaling. In normal physiology, the pulmonary vasculature provides a low pulmonary vascular resistance (PVR) under high blood flow with minimal elevations of pulmonary artery pressure (PAP). [3] Endothelial cells are exposed to tangential shear stress and circumferential wall stretch by the blood flow through the pulmonary vasculature. [4] Changes in endothelial shear stress result in a cellular signaling cascade and trigger inflammation with an increased production of reactive oxygen species (ROS) and nitric oxide (NO) [5], which may result in oxidative injury. Once reperfusion of the transplanted allograft occurs, ROS and pro-inflammatory cytokines activate neutrophils and upregulation of cell-surface adhesion molecules on the endothelial side of the lung occurs. Disruption of alveolo-capillary membrane results in increased microvascular permeability (Pμvasc), increased PVR, impaired oxygenation and eventually pulmonary edema, referred to

as IRI. [6] This mechanism also explains why alteration in shear stress and ischemia result in more lung injury, rather than anoxia. [7-8]

Animal models provide a broad study field to verify clinical findings and are the cornerstone of translational research. The single left porcine LTx model is commonly used to study the early stages of lung transplantation (donor, preservation and reperfusion), [9-11] In most of the published models, however, the native right lung is not clamped after implantation when testing functional performance of the left allograft. An important reason is the risk of acute right ventricular (RV) failure following clamping of the right pulmonary artery (PA). [12-14]

However, under these circumstances, it is difficult to assess the function of the transplanted lung alone and selective reperfusion to the left allograft is never monitored. Studies with (partial) clamping of the right PA will help to better understand physiologic changes during bilateral LTx when both lungs are implanted in a sequential way without support by extracorporeal techniques providing a right to left oxygenated shunt. Also, these clamping studies might contribute to the knowledge of intra-operative events where the standard procedure is to transplant the left and the right lung in a sequential setting.

In this study, we aimed to develop a porcine left LTx model with an innovative approach to c We hypothesized that IRI in the allograft is more severe in a high flow than in a low flow reperfusion model.

Materials And Methods

This experimental porcine study (topig20 pigs, Zoötechnisch centrum KU Leuven, Lovenjoel, Belgium) was approved by the Ethics Committee on Animal Research KU Leuven (P011/2018). All animals received human care in accordance with “Principles of Laboratory Animal care,” formulated by the National Society for Medical Research and “Guide for the Care and Use of Laboratory Animals,” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health, USA (NIH Publication No. 86-23, revised 1996).

Study groups

24 domestic pigs were divided into two groups: high flow (HF) (n = 6 x donor + recipient) and low flow (LF) (n = 6 x donor + recipient) group. In both groups, lungs were harvested after cold antegrade flush in the donor animal. After 24 hours cold ischemia by storage on 4°C ice, the left graft was transplanted in a recipient animal. In the HF group, the right PA to the native lung was isolated, encircled with an umbilical tape, and partially clamped after the first 2 hours (target flow 40 – 60% of total cardiac output) to achieve a higher flow to the transplanted lung. In the LF group, the right PA was not clamped. Hemodynamic parameters and gas exchange were measured during 6 hours of reperfusion in both groups.

Donor procedure

After sedating the donor animal with an intramuscular injection of 5 mg/kg Zoletil 100 (Virbac, Carros, France) and 3 mg/kg Xyl-M 2% (VMD, Arendonk, Belgium), anesthesia was maintained with 10 mg/kg/h

propofol, 20 µg/kg/h fentanyl and intermittent boli of pancuronium 2 mg for muscle relaxation. Animals were intubated with a 7.0 mm endotracheal tube and ventilated (Aestiva 3000; GE Healthcare Europe GmbH, Little Chalfont, UK) with a tidal volume (TV) of 8 ml/kg, positive end-expiratory pressure (PEEP) of 5 cmH_2O and FiO_2 of 30%. Respiratory rate (RR) was adjusted to end-tidal carbon dioxide levels ($ETCO_2$) (45–55 mmHg). A lateral right neck incision was made to access the right carotid artery for invasive monitoring of arterial blood pressure (ABP). Median sternotomy was performed. Prior to cardiac arrest induced by aortic cross clamping, all animals were anticoagulated with 300 IU/kg heparine. The thymus was resected and the pericardium opened. Inferior (IVC) and superior (SVC) caval veins were isolated, and the aorta was separated before PA cannulation. After ligation of SVC and IVC and aortic-cross clamp, grafts were flushed antegrade via the PA cannula with 2 liters (L) of cold (4°C) buffered OCS®-solution (Transmedics, Andover, MA, USA). Heart-lung block was harvested and the trachea was double-clamped with lungs being inflated and maintaining an airway pressure of 15 cmH2O. On the back table a retrograde cold flush with 800 mL buffered OCS-solution was performed via the pulmonary veins following excision of the heart. Lungs were placed in two plastic bags and stored in OCS® solution at 4 °C for 24 hours.

Recipient procedure

After anesthetizing the recipient animal and maintaining anesthesia as described above for the donor procedure, a deep venous catheter was inserted in the internal jugular vein as well as an arterial catheter in the carotid artery. A mini-laparotomy was performed to insert a bladder catheter. Animal body temperature was monitored with a rectal probe. The pig was turned to a right lateral decubitus position and a left thoracotomy in the 4^{th} intercostal space was performed. All animals were heparinized with 300 IU/kg. After dissection of the pulmonary ligament and ligation of the left hemi-azygos vein, a left pneumonectomy was performed. PA pressure (PAP) and left atrium (LA) pressure (LAP) were monitored with invasive catheters in the common PA and LA. Pulmonary vascular resistance (PVR) was calculated by the formula: 80*PAP-LAP divided by PA flow and was expressed in dyn/s/cm $^{-5}$. PA blood flow was measured by flowmeters (Transonic Systems Inc.®, Ithaca, NY) encircled around the common PA and around the left PA anastomosis after left lung implantation to capture the total cardiac output as well as the blood flow to the graft (figure 1). The left donor lung was transplanted by three anastomoses in the following order: 1. bronchus with a running 4-0 PDS suture on the posterior and anterior walls; 2. LA cuff with a running 5-0 prolene suture; and 3. PA with a running 5-0 prolene suture. After opening clamps, the graft was reperfused and the animal was monitored for 6 hours. Whenever necessary, norepinephrine (Levophed, Pfizer Inc., US) was administrated intravenously for vasopressor support to maintain mean ABP above 50 mmHg starting with an initial dose of 8-12 mcg/min continuously. Lactate ringer was added (8 ml/Kg/h) to maintain fluid balance. At the end of the experiment, animals were sacrificed by aortic clamping.

In the HF group, the right PA was partially clamped after 2 hours reperfusion to achieve a flow to the transplanted left allograft >50% of CO.

Sampling

Upon reperfusion and during the monitoring period, blood samples were taken hourly from the carotid artery, PA, and right and left pulmonary veins (RPV, LPV) to monitor gas exchange. Differential blood gases from RPV and LPV allowed to discriminate the oxygenation capacity of the right native versus the left transplanted lung. At the end of the experiment, a bronchoalveolar lavage (BAL) with two times 20 cc saline 0.9% was performed in the left lower lobe and the supernatant was analyzed with a porcine multiplex enzyme-linked immuno-sandwich assays (ELISA) kit for measurement of interleukin-6 (IL-6) and interleukin-8 (IL-8) levels according to the manufacturer's protocol (R&D Systems, Inc. Minneapolis, MN, USA) with lower limits of quantification (LLOQ): 4.69 pg/ml for IL-6 and 31.25 pg/mg for IL-8.

Porcine multiplex ELISA according to the manufacturer's protocol (ThermoFischer, Scientific, Vienna, Austria) were performed on plasma samples, collected from each animal at baseline and at the end of the experiment for cytokine analysis, including interferon-γ (IFN-γ), interferon-α (IFN-α), interleukin-1beta (IL-1β), interleukin-10 (IL-10), interleukin-12p40 (IL-12p40), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) with lower limits of quantification (LLOQ): 0.6 pg/ml for IFN-γ, 4.5 pg/ml for IFN-α, 3.2 pg/ml for IL-1β, 18 pg/ml for IL-10, 30 pg/ml for IL-12p40, 1.5 pg/ml for IL-4, 5.9 pg/ml for IL-6, 16 pg/ml for IL-8, 6.5 pg/ml for TNF-α.

Lung biopsies were taken from the right and left lower lobe (RLL, LLL) from the recipient at the end of the experiment and from RLL of the (unused) donor lung at the end of the experiment and subsequently formalin fixed, paraffin embedded, and hematoxylin-eosin stained. Biopsies were scored for presence of interstitial widening, capillary congestion, intra-alveolar edema, hemorrhage, neutrophils in septa and in alveoli, and eosinophils in septa by a pathologist blinded for experimental groups. Also, biopsies for wet-to-dry weight (W/D) ratio calculation (after 72 h in the oven at 80 °C) were taken to quantify lung edema. [15]

Statistical analysis

All data are described as median with interquartile range (IQR) (25% QI–75% QI) in GraphPad Prism 8 (GraphPad Software Inc, La Jolla, CA, USA). Values were compared between time points (T0-T6) and between both study groups using 2-way ANOVA or Mann-Whitney U-test and post-hoc multiple comparison test Sidak (x). A p-values of ≤ 0.05 was considered significant.

Results

Functional assessment of pulmonary graft during reperfusion

Physiological parameters assessed over the 6 h reperfusion period are presented in table 1 and figure 2.

Cardiac output was comparable over time between both groups (p=0.32) (figure 2A).

A greater blood flow to the transplanted left lung was achieved in the HF (1.41 L) group over the 6 h vs. the LF (0.49 L) group (p=0.0005) with significant differences after 5 h (p=0.03) and 6 h of reperfusion (p=0.0002) (figure 2B). The blood flow through the right native lung (not transplanted lung) was significantly higher in the LF group (3.40 L vs 2.77 L) over the 6 h (p=0.04) and the post hoc analysis demonstrated a significant difference after 6 hours reperfusion (p=0.04) (figure 2C).

P/F ratio of LPV tended to be lower (p=0.08) in the HF group (229.1 mmHg) vs. the LF group (308.9 mm Hg) over the 6 h reperfusion period. Looking at the P/F ratio of RPV, there was no significant difference between both groups (392.6 mmHg in LF vs. 376.4 mmHg in HF, p=0.60) (figures 2D-F).

Mean PAP was not different in the HF group vs. the LF group (34.6 mmHg vs. 29.8 mmHg) (p=0.16) over the time of 6 h reperfusion (figure 2G). Compliance of the lung did not show any significance (p=0.66) between the groups (37.05 ml/cm H_2O in LF vs 35.90 ml/cm H_2O in HF group) (figure 2H).

Isolated PVR of the native right lung was significantly higher (p=0.03) in the HF group (581.2 dyn·s/cm^{-5} in LF vs. 946.9 dyn·s/cm^{-5} in HF group) (figure 2I). Moreover, the post hoc analysis revealed a significant difference after 6 h reperfusion (p=0.04).

Assessment at the end of reperfusion

All data for LF and HF group at the end of the 6 h reperfusion period (T6) are depicted in table 1. Significantly higher median flow to the transplanted lung was achieved in the HF group compared to the LF group: 2.41 L/min (54.6% of CO) vs. 0.49 L/min (13.1% of CO) (p=0.01). Furthermore, there was a significant difference between blood flow to the right native lung in the LF group compared to the HF group (3.35 L/min, 86.9% of CO vs. 1.7 L/min, 45.4% of CO, p=0.01). There was a significant difference in PVR between the left and right lung in both groups (2763 dyn*s/cm^5 versus 999.7 dyn*s/cm^5, p=0.01). Also, PVR of the right lung was significantly different between both groups (p=0.009).

In contrast, there was no significant difference in mPAP at T6 between both groups (p=0.22). Also, lung compliance was not different between the LF and HF group (p=0.73).

After 6 h reperfusion W/D of right native lung (p=0.49) and left transplanted lung were similar (p>0.99) (figure 3A).

Immunological evaluation

Porcine multiplex ELISA analysis of the plasma at the end of the experiment between LF and HF group for the cytokines IFN-□ (p= 0.33), IFN-□ (p= 0.10), IL-1β (p= 0.59), IL-10 (p= 0.70), IL-12p40 (p= 0.82), IL-4 (p >0.99), IL-6 (p= 0.18), IL-8 (p= 0.67), and TNF-□ (p= 0.32) did not show any differences between both groups (table 2). Similarly, no significant differences were demonstrated in the single cytokine ELISA analysis of BAL samples between the LF and HF group (IL-6, p= 0.23, IL-8, p= 0.076).

Histology

Histologic abnormalities were equally present in the LF and HF group (figure 3B-E) when comparing the right native and transplanted left lung in each group.

Although, in the HF group, the native right lower lobe (RLL) showed prominent capillary congestion and presence of neutrophilic infiltration in the septa compared to the LLL of the LF group with mild capillary congestion and mild septal neutrophilic infiltration without presence of intra-alveolar neutrophils. The transplanted left lower lobe (LLL) of the HF group shows presence of capillary congestion, prominent intra-alveolar edema and presence of septal and intra-alveolar neutrophilic infiltration compared to the LLL of the LF group with mild capillary congestion, presence of septal neutrophilic infiltration, intra-alveolar edema and intra-alveolar neutrophils.

In table 3 the results of histological scoring of lung biopsies are shown. Comparison of the native (right lower lobe) and transplanted lung in the HF group did not show any differences. In the LF group though, the presence of neutrophils in septa ($p=0.02$) and neutrophils intra-alveolar ($p=0.01$) were significantly increased in the transplanted lung compared to the native right lung.

Discussion

In this experimental study, we have demonstrated that a higher PA blood flow through the transplanted lung results in a lower P/F ratio which could be an indicator for a more severe grade of clinical PGD. To the best of our knowledge, we are the first to selectively assess ischemia-reperfusion injury in the transplanted left lung graft and to investigate the impact of

the PA flow through the transplanted allograft. To avoid acute right ventricular failure, the right PA was only partially clamped as the non-dilatable suture line of the PA anastomosis of the left transplanted lung may create a relative obstruction and therefore cause an increased right ventricular afterload. Our technique to perform a wider PA anastomosis in our porcine LTx model was previously described. [16]

Oxygenation is an important determinant to assess IRI and PGD after transplantation. In our model, systemic P/F values were higher than 300 mmHg in both groups, which would indicate that no PGD developed. However, the mean P/F ratio of LPV in the HF group was 229.1 mmHg and indicates PGD compared to P/F ratio of 308.9 mmHg of LPV in the LF group.

These findings indicate that it is necessary to evaluate the oxygenation capacity of both native and transplanted lung separately and also that a high pulmonary flow through the allograft might be necessary to develop important IRI.

The impact of PA *flow* and physiological changes in the graft after reperfusion and in the early postoperative period remains unclear. However, compared to systemic organs, cessation of blood flow results in hypoxia, except in the lungs where adequate tissue oxygenation can be maintained through ventilation only. [17] The terms “mechanotransduction, mechanosensing, mechanosignaling” are referring to a signaling cascade sensed by the pulmonary endothelium when blood flow ceases. [18] Endothelial

mechanotransduction by abrupt cessation of blood flow to understand the role of ischemia-mediated ROS in signaling has been studied by other groups. [18-23] Al-Mehdi and colleagues demonstrated in a rat model that a low perfusate flow rate can prevent activation of the loss of shear stress signaling cascade (mechanotransduction). [24]

Other early attempts to study blood flow to each lung with the *measurement of PAP* and LAP were performed in an anesthetized dog in 1956, concluding that vascular resistance is affected by LAP and PAP; and by blood flow. [25] Another early study in mongrel dogs demonstrated that change of blood flow through the PA results in small, but prompt, changes in PAP. Moreover, they concluded that PVR is influenced by flow and increases with decreasing flows. [26]

In the HF group in our study, we observed a trend for lower PVR compared to the LF group but no significance. Our results also demonstrated that isolated PVR of the left lung did not change with any significant increase in flow, compared to the isolated PVR of the right native lung, whereas we observed a significantly greater PVR in the high flow group. Despite partial clamping of the right PA to achieve a greater pulmonary flow through the allograft, all animals survived the 6 h reperfusion time and the partial PA clamping did not result in right ventricular failure right heart failure. [27] In both groups cardiac output was similar and we did not observe any significant difference during the 6 h reperfusion. Therefore, no ventricular right heart failure was observed in our study.

Also, general *inflammatory markers* such as IFN- α, IFN- γ, IL-1β, IL-10, IL-12p40, IL-4, IL-6, IL-8, TNF-α measured in the plasma at the beginning and in the end of the experiment, were increased in both groups, showing activation of the innate immune system, without differences between study groups. In this study measurements of immunologic markers, reflecting lung injury, were measured at a very early time point. Hamilton et al. describe biomarkers associated with PGD within the first 72 hours post-LTx. There is a clear peak of biomarkers reflecting lung injury between 8 and 24 hours after LTx. [28] Therefore, it is questionable how much lung injury can already be observed after 6 hours reperfusion like in our porcine LTx model. A 24-hour cold ischemia model was used. There was no difference in water accumulation observed between both groups using W/D ratio as a gold standard for quantifying lung edema. [15] A reasonable explanation for our observation could be related to a too short reperfusion time. In our model lungs were reperfused for 6 hours and the animal was sacrificed at the end of the experiment. In clinical practice, PGD is assessed for 72 hours post-LTx according to the guidelines of the consensus group statement of the ISHLT. [1]

Our investigations using porcine lungs demonstrated that injury proven in histological samples occurs in the native right and transplanted lung within the first 6 hours as a result of IRI. Within the HF group histological samples showed a greater injury under higher flow conditions. Interestingly not only the transplanted left lung showed histological injury, also the right native lung was damaged as reflected in the LF group as mild capillary congestion and mild septal neutrophilic infiltration without presence of intra-alveolar neutrophils. In the HF group histological injury of the right native lung showed shows prominent capillary congestion and presence of neutrophilic infiltration in the septa.

The remaining question regarding this observation is whether injury of the right native lung was caused by ventilation, reperfusion injury, spillover of toxic agents from the left lung, or due to systemic stress response to the transplantation procedure. Probably all these mechanisms together apply. This should be further investigated in a transplantation setting.

It is well established that the mode of invasive ventilation during the early phase of reperfusion can aggravate IRI. [29] To reflect this in our model, lungs are ventilated with a TV of 8 ml/kg and PEEP of 5 cmH_2O during the baseline procedure; which was reduced TV to 2/3 after pneumonectomy and during implantation. TV is then switched back to 8 ml/kg upon reperfusion. The responsible mechanism for ventilator-induced lung injury (VILI) is mechanical stress induced by shear stress and should be considered during a transplantation in favor of the right native lung.

Limitations

Our study serves as a preclinical model to study PGD. A potential limitation of our study is that we perform a single lung transplantation. This is because bilateral LTx in pigs is not feasible due to its anatomical variables compared to humans. A single right LTx may have been better because of a larger vascular bed compared to the left. In addition, complete right hilar clamping is not feasible in a left lung transplanted pig for a 6-hour survival model because of the high incidence of acute right heart failure. We realize that the absolute number of animals in each group is relatively low, especially to perform reliable statistical comparison. Nevertheless, we present a reproducible model with low variability in both groups. We believe that our investigations in 12 animals were enough to confirm the validity of our model.

Conclusion

Porcine single-lung transplantation models remain demanding, but the setting is feasible.

In this model we could demonstrate that the P/F ratio in the transplanted left lung is dependent on PA flow. Our innovative approach to control blood flow towards the transplanted lung and to monitor the function of the right native lung separately allows an in-depth study physiology and treatment of PGD.

Importantly, a porcine left single-lung transplantation setting is valuable and contributes to a better understanding of physiological changes after LTx.

Abbreviations

ABP	Arterial blood pressure
BAL	Broncho-alveolar lavage
CO	Cardiac output
ELISA	Enzyme-linked immuno sandwich assays

$ETCO_2$	End-tidal carbon dioxide levels
HF	High flow
ICU	Intensive care unit
IFN-γ	Interferon-γ
IFN-γ	Interferon-γ
IL-1 β	Interleukin-1beta
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12p40	Interleukin-12p40
IVC	Inferior vena cava
IRI	Ischemia-reperfusion injury
L	Liters
LA	Left atrium
LAP	Left atrial pressure
LF	Low flow
LLL	Left lower lobe
LLOQ	Lower limits of quantification
LPV	Left pulmonary vein
LTx	Lung transplantation
Pμvasc	Microvascular permeability
NO	Nitric oxide
NADPH	Nicotinamide adenine dinucleotide phosphate

NF-κB	Nuclear factor-κB
NOS	Nitric oxide synthases
P/F ratio	P_aO_2/F_iO_2 ratio
PA	Pulmonary artery
PAP	Pulmonary artery pressure
PEEP	Positive end-expiratory pressure
PGD	Primary graft dysfunction
PH	Pulmonary hypertension
PVR	Pulmonary vascular resistance
RLL	Right lower lobe
ROS	Reactive oxygen species
RPV	Right pulmonary vein
RR	Respiratory rate
RV	Right ventricular
SVC	Superior vena cava
TNF-	Tumor necrosis factor-
TV	Tidal volume
VILI	Ventilation-induced lung injury
W/D	Wet-to-dry ratio

Declarations

Ethical approval and consent to participate

This experimental porcine study (topig20 pigs, Zoötechnisch centrum KU Leuven, Lovenjoel, Belgium) was approved by the Ethics Committee on Animal Research KU Leuven (P011/2018). All animals received human care in accordance with “Principles of Laboratory Animal care,” formulated by the National Society for Medical Research and “Guide for the Care and Use of Laboratory Animals,” prepared by the

Institute of Laboratory Animal Resources and published by the National Institutes of Health, USA (NIH Publication No. 86-23, revised 1996).

Consent for publication

All authors have read and approved the final manuscript.

Availability of data and materials

The authors confirm that the data suppporting the findings of this study are available within the article.

Competing interests

There are no competing interests.

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Authors' contributions

A.E.F., M.O., B.S., J.K., T.H. and S.O. performed the research work and data collection. A.E.F. and S.E.V. performed the statistical analysis. A.V. scored all histology samples. S.C., D.S., A.E.F. and S.E.V. performed and interpreted the multiplex analysis. S.E.V., G.M.V., R.V. A.P.N., B.M.V., and D.E.V.R. contributed to the conception and design of the study and did the final revision of the article. All authors read and approved the final article.

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Tables

Table 1 Outcome parameters at the end of reperfusion in low vs high flow group (T6)

	LF	HF	p-value (p<0.05)
number of pigs, n	**6**	**6**	
Parameters at T6			
CO, L/min	3.8 (3.3 - 5.1)	4.2 (3.4 - 5.1)	0.80
flow left PA, L/min, %	0.5 (0.4 - 0.9), 13.1	2.4 (1.8 - 2.7), 54.7	**0.01**
flow right PA, L/min, %	3.4 (2.7 - 4.2), 86.9	1.7 (1.5 - 2.5), 45.3	**0.01**
mPAP, mmHg	30.5 (22.0- 39.0)	38.5 (29.5 - 45.0)	0.22
lung compliance, ml/cm H2O	35.0 (27.8 - 47.3)	35.5 (24.3 - 41.8)	0.73
PVR, dyn·s·cm -5	482.4 (210.6 - 751.7)	550.2 (441.5 - 728.6)	0.70
PVR left lung, dyn·s·cm -5	2763.1 (1598 - 5626)	999.7 (729.8 - 1479)	**0.01**
PVR right lung, dyn·s·cm -5	585.7 (229.7 - 951.6)	1273 (1045 - 1561)	**0.01**
W/D donor RLL	5.6 (5.5 - 5.7)	5.6 (5.5 - 5.7)	0.91
W/D native RLL	6.7 (± 0.5)	6.8 (± 0.3)	0.49
W/D transplanted LLL	7.9 (± 0.4)	8.3 (± 0.8)	>0.99

Data are expressed as median (25%–75% interquartile range); and Mann Whitney was used for comparing the two groups; CO, cardiac output; PA pulmonary artery; pO_2, partial pressure of oxygen; LPV, left pulmonary vein; RPV, right pulmonary vein; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; T6, after 6 hours reperfusion; W/D, wet-to-dry weight ratio; RLL, right lower lobe; LLL, left lower lobe; LF, low flow; HF high flow;

Table 2 Cytokines measurements in plasma of low vs high flow group

Cytokines	LLOQ (pg/ml)	LF	HF	p-value
IFN-alpha	0.6	0.5 (0.3 - 3.6)	0.9 (0.6 - 1.4)	0.33
IFN-gamma	4.5	5.4 (4.6 - 12.9)	13.9 (10.4 - 46.9)	0.10
IL-1beta	3.2	11.3 (5 - 299.1)	17.4 (8.3 - 32.9)	0.59
IL-10	18	56 (24.7 - 535.5)	73.5 (55.2 -89.2)	0.70
IL-12p40	30	510.1 (221.6 - 720.3)	265.5 (214.6 - 901.6)	0.82
IL-4	1.5	2.5 (0.7 - 5.4)	1.9 (1.8 - 2.5)	>0.99
IL-6	5.9	73.5 (36.9 - 745)	150.4 (102.1 - 260.2)	0.18
IL-8	16	43.1 (22.1 - 1172)	30.4 (23.2 - 35.4)	0.67
TNF-alpha	6.5	101.7 (3.2 - 1139)	3.2 (3.2 - 85.7)	0.32

Cytokine measurements for the cytokines: interferon-□(IFN-□), interferon-□ (IFN-□), interleukin-1β (IL-1β), interleukin-10 (IL-10), interleukin-12p40 (IL-12p40), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-□ (TNF-□) were not significant in the low vs. high flow group. Data are expressed as median (25%–75% interquartile range); and Mann Whitney test was used for comparing the two groups; LLOQ, lower limit of quantification; pg/ml, picogram/milliliter; LF, low flow; HF, high flow;

Table 3 Lung biopsies from RLL and LLL in LF and HF group

Group	LF	HF		LF	HF		LF			HF		
Location	LLL		p-value	RLL		p-value	RLL	LLL	p-value	RLL	LLL	p-value
Pathological features												
Interstitial widening, grade 0-3	1.5 (1 - 2.25)	1 (1 - 1.25)	0.42	1.5 (1 - 2.25)	1 (1 - 1.25)	0.42	1.5 (1 - 2.25)	1.5 (1 - 2.25)	>0.99	1 (1 - 1.25)	1 (1 - 1.25)	>0.99
Capillary congestion, grade 0-2	2 (2-2)	2 (1.75-2)	>0.99	2 (1.75 - 2)	2 (0.75 - 2)	0.73	2 (2 - 2)	2 (1.75 - 2)	>0.99	2 (0.75 - 2)	2 (1.75 - 2)	0.73
Intraalveolar edema, grade 0-2	2 (0.75 - 2)	1 (0 - 1.25)	0.21	0 (0 - 1.25)	0 (0-0)	0.45	0 (0 - 1.25)	2 (0.75 - 2)	0.13	0 (0 - 0)	1 (0 - 1.25)	0.06
Hemorrage, grade 0-2	1 (0 - 2)	1.5 (1 - 2)	0.45	2 (0.75 - 2)	1 (0.75 - 2)	0.54	2 (0.75 - 2)	1 (0 - 2)	0.47	1 (0.75 - 2)	1.5 (1 - 2)	0.68
Neutrophils in septa, grade 0-3	3 (2 - 3)	2 (2- 3)	0.57	1.5 (1 - 2)	1.5 (1 - 2)	>0.99	1.5 (1 - 2)	3 (2 - 3)	**0.02**	1.5 (1 - 2)	2 (2 - 3)	0.08
Neutrophils intraalveolar, grade 0-2	2 (1.75 - 2)	1.5 (0 - 2)	0.30	0.5 (0 - 1)	0 (0 - 1.25)	>0.99	0.5 (0 - 1)	2 (1.75 - 2)	**0.01**	0 (0 - 1.25)	1.5 (0 - 2)	0.37

Biopsies were scored for presence of interstitial widening, capillary congestion, intra-alveolar edema, hemorrhage, neutrophils in septa, and neutrophils intra-alveolar by a pathologist blinded for experimental groups. Gradings were from 0 (considered as absent) to grade of severity (1-3); LF, low flow; HF, high flow; LLL, left lower lobe; RLL, right lower lobe; Data are expressed as median (25%–75% interquartile range); and Mann Whitney test was used for comparing the two groups;

Figures

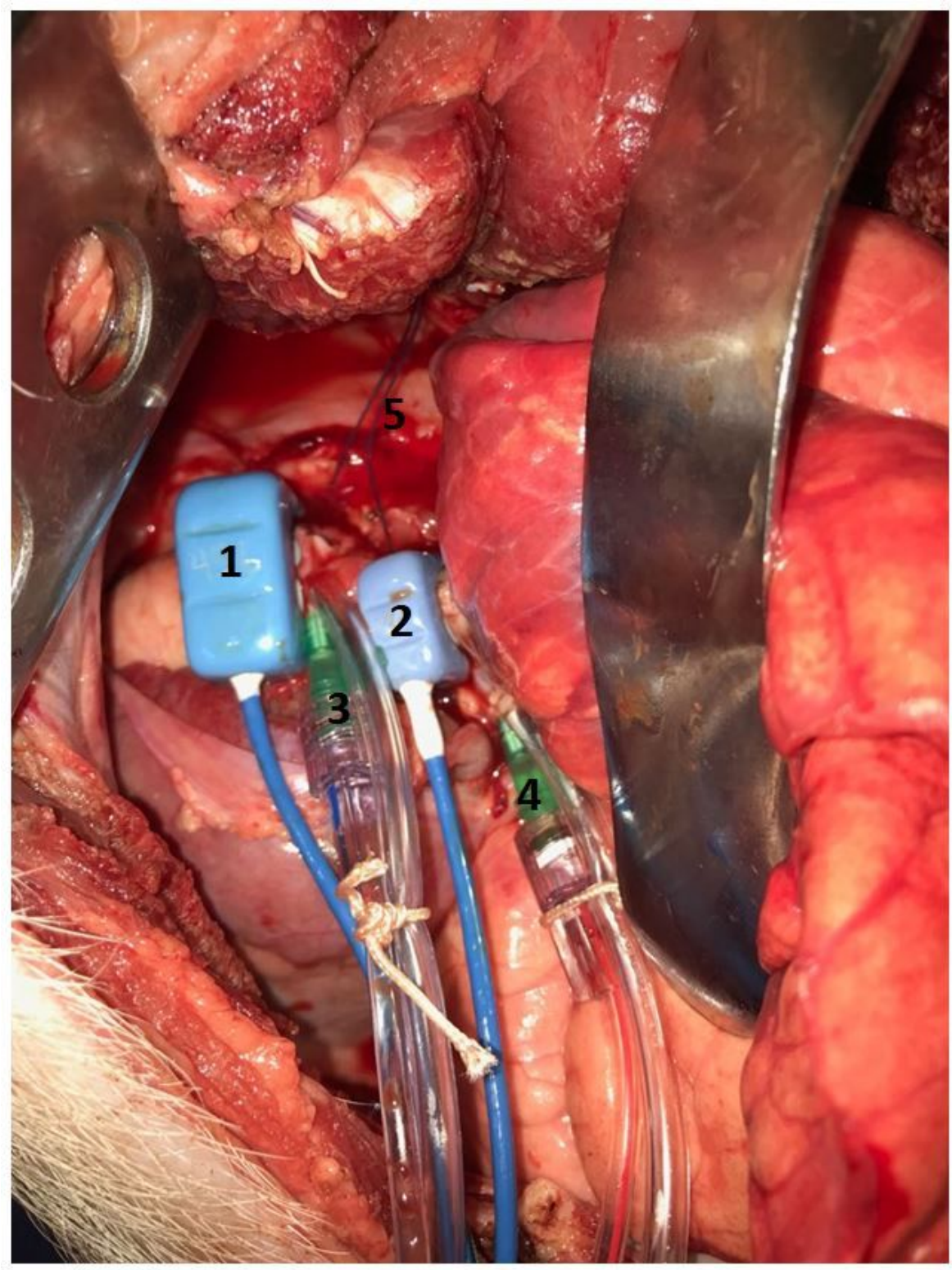

Figure 1

Illustration of monitoring in the recipient Figure 1 demonstrates the monitoring in the recipient. An invasive catheter was inserted in the carotid artery, jugular vein, left atrium and pulmonary artery for invasive monitoring of different hemodynamic parameters. All recipients were intubated with an endotracheal tube. Around the main and left pulmonary artery flow probes were placed to measure the cardiac output. 1: flow probe around common pulmonary artery; 2: flow probe around left pulmonary

artery; 3: invasive catheter in pulmonary artery; 4: invasive catheter in left atrium; 5: isolated right pulmonary artery;

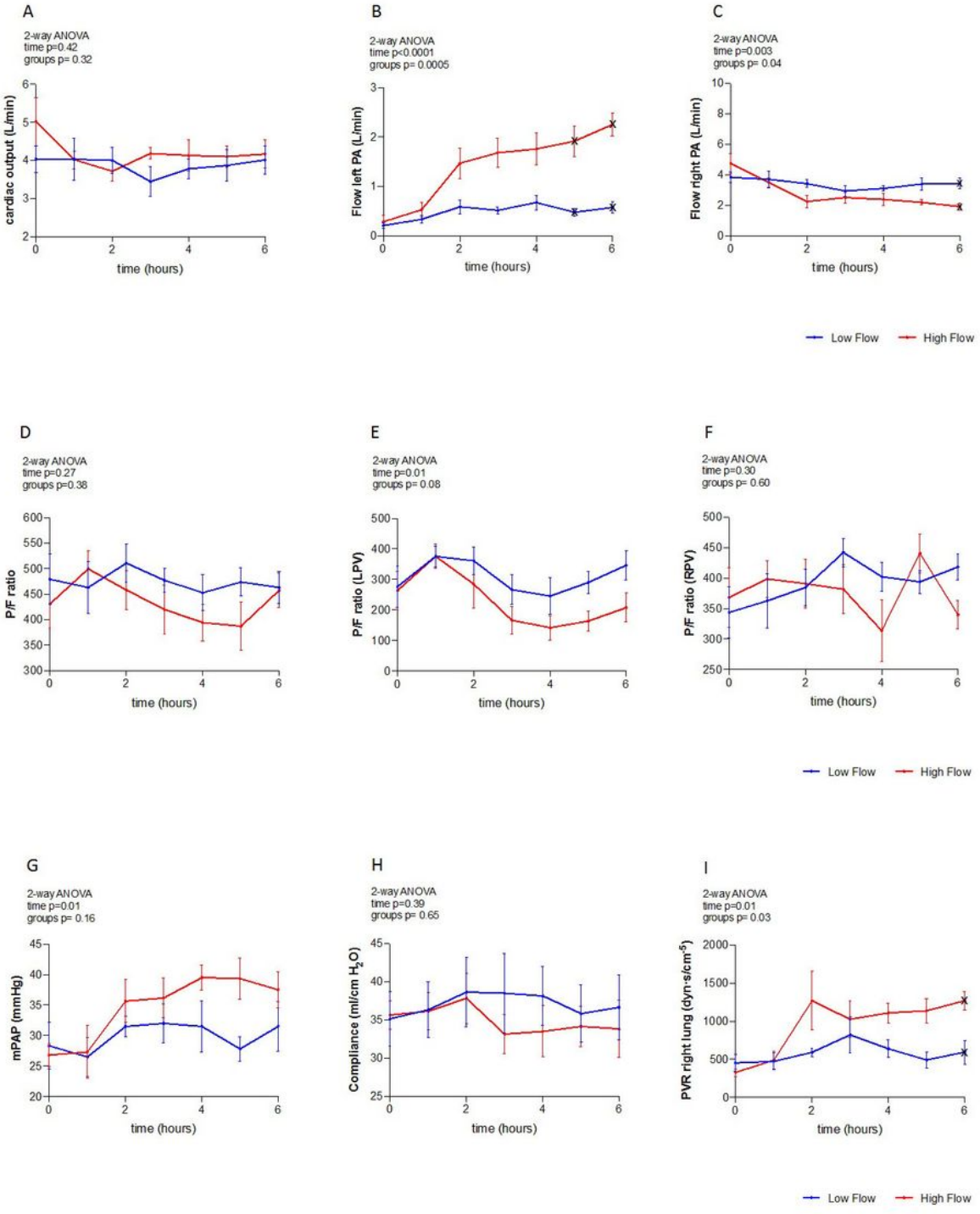

Figure 2

(A-I) Parameters during reperfusion Figure 2A-C Assessment of hemodynamic parameters during 6h reperfusion. CO and flow to the left lung were measured, flow through the right PA was calculated; All data are depicted as median ± IQR analyzed with repeated measures two-way ANOVA (A-C) and post-hoc

multiple comparison test Sidak (x). Time is 6 h reperfusion; CO, cardiac output; PA pulmonary artery; Figure 2D-F Assessment of oxygenation; blood gases samples were taken from carotid artery (P/F ratio), left pulmonary vein (LPV) and right pulmonary vein (RPV). All data are depicted as median ± IQR analyzed with repeated measures two-way ANOVA (A-C) and post-hoc multiple comparison test Sidak (x). Time is 6 h reperfusion; pO2, partial pressure of oxygen; Figure 2G-I Assessment of hemodynamic parameters and compliance during 6h reperfusion; mPAP was measured. Compliance of the lung in the low and high flow lung was measured. PVR of right lung was calculated with: 80*(PAP-LAP)/CO; All data are depicted as median ± IQR analyzed with repeated measures two-way ANOVA (A-C) and post-hoc multiple comparison test Sidak (xTime is 6 h reperfusion; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance;

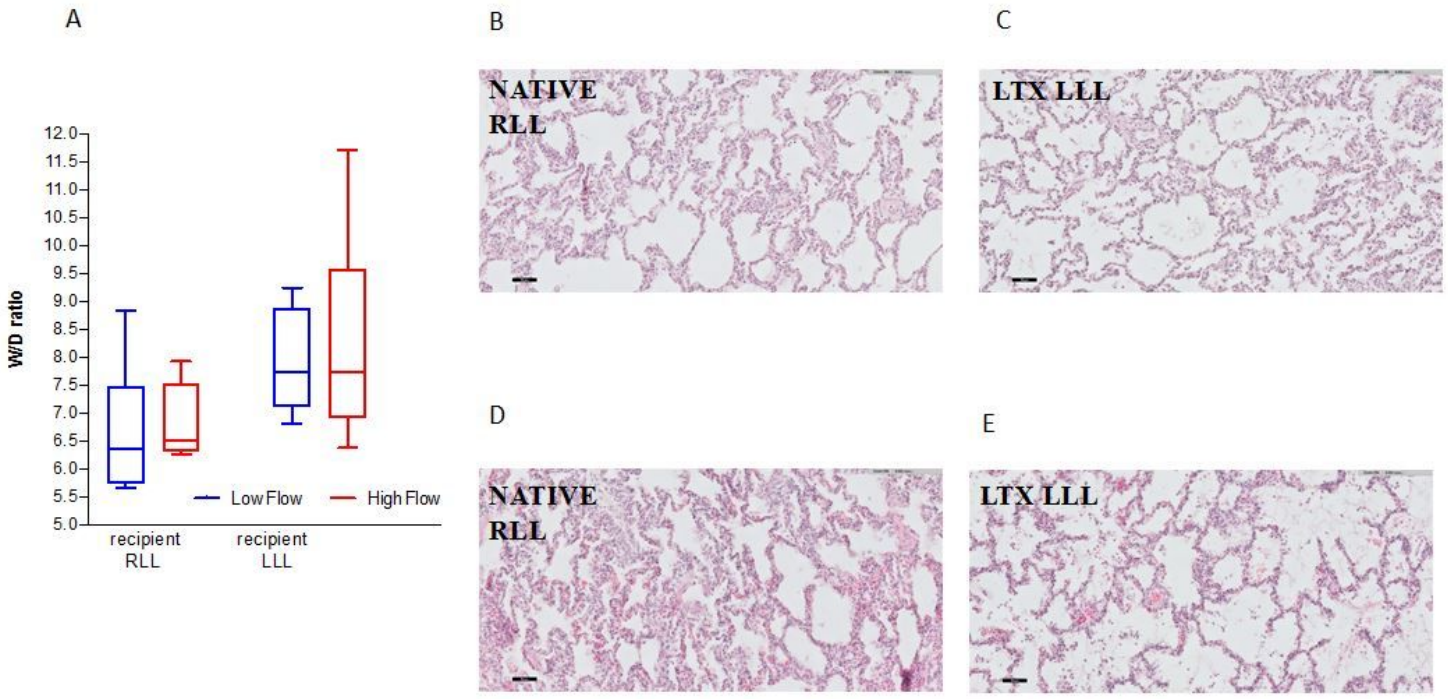

Figure 3

(A-E) Histology Figure 3AThe W/D ratios were assessed of lung biopsies at the end after 6 h reperfusion. No significant difference was observed between the low vs. high flow group in the right native lug ($p=0.49$) and the left transplanted lung ($p<0.99$). Data were analyzed with Mann-Whitney test; W/D, wet-to-dry weight ratio; RLL, right lower lobe; LLL, left lower lobe; Figures 3B-E Left (B). The native right lower lobe (RLL) of the low flow (LF) group shows mild capillary congestion and mild septal neutrophilic infiltration without presence of intra-alveolar neutrophils. Right (C). The transplanted left lower lobe (LLL) of the low flow (LF) group shows mild capillary congestion, presence of septal neutrophilic infiltration, intra-alveolar edema and intra-alveolar neutrophils. Figure 3D-E Left (D). The native right lower lobe (RLL) of the high flow (HF) group shows prominent capillary congestion and presence of neutrophilic infiltration in the septa. Right (E). The transplanted left lower lobe (LLL) of the (HF) group shows presence of

capillary congestion, prominent intra-alveolar edema and presence of septal and intra-alveolar neutrophilic infiltration.