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Impact of Anastomosis Time during Lung Transplantation on Primary Graft Dysfunction

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ABBREVIATIONS:
AT, anastomosis time
CIT, cold ischemic time
CLAD, chronic lung allograft dysfunction
DBD, donation after brain death
DCD, donation after circulatory death
DLTx, double lung transplantation
DWIT, donor warm ischemic time
ECLS, extra-corporeal life support
EVLP, ex-vivo lung perfusion
ICU, intensive care unit
IRI, ischemia-reperfusion injury
LTx, lung transplantation
PAH, pulmonary arterial hypertension
PGD, primary graft dysfunction
PGD3, primary graft dysfunction grade 3

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ABSTRACT

Primary graft dysfunction (PGD) is a major obstacle after lung transplantation (LTx), associated with increased early morbidity and mortality. Studies in liver and kidney transplantation revealed prolonged anastomosis time (AT) as independent risk factor for impaired short- and long-term outcomes. We investigated if AT during LTx is a risk factor for PGD. In this retrospective single-center cohort study we included all first double lung transplantations (DLTx) between 2008-2016. The association of AT with any PGD grade 3 (PGD3) within the first 72 hours post-transplant was analyzed by univariable and multivariable logistic regression analysis. Data on AT and PGD was available for 427 patients of which 130 (30.2%) developed PGD3. AT was independently associated with the development of any PGD3 ≤72 hours in uni- (OR per 10min 1.293, 95%CI (1.136-1.471), p<.0001) and multivariable (OR 1.205, 95%CI (1.022-1.421), p=0.03) logistic regression analysis. There was no evidence that the relation between AT and PGD3 differed between lung recipients from donation after brain death versus donation after circulatory death donors. This study identified AT as independent risk factor for development of PGD3 post-LTx. We suggest that the implantation time should be kept short and the lung cooled to decrease PGD-related morbidity and mortality post-LTx.

MAIN BODY TEXT

1. INTRODUCTION

Lung transplantation (LTx) is the ultimate treatment option for selected patients with end-stage pulmonary disease, improving survival and quality of life[1]. Despite significant improvements in organ preservation, surgical techniques, peri-operative care and immunosuppression over the past decades, the success of LTx is still hampered by several challenges like limited donor availability[2], ischemia-reperfusion injury (IRI)[3,4], primary graft dysfunction (PGD)[5,6] and chronic lung allograft dysfunction (CLAD)[7,8].

Lung IRI provokes multiple cellular mechanisms, resulting in epithelial and endothelial cell injury, innate immune activation, an inflammatory cascade and cross-talk with the adaptive immunity[9,10]. This cascade contributes to PGD development, a severe form of acute lung injury occurring in the first 72 hours post-transplant, which is a risk factor for increased morbidity and mortality in the early post-transplant period [11].

Several risk factors, related to the transplant process have been identified to be associated with inferior LTx outcome, including prolonged cold and total ischemic time[12,13,14], timing of allograft implantation[15] and the use of per-operative extra-corporeal life support (ECLS)[12,16]. In contrast to kidney and liver transplantation, donor agonal phase and donor warm ischemic time (DWIT) are not correlated with early survival after LTx from donation after circulatory death (DCD) donors[17]. This can be attributed to the unique oxygen reserve capacity of the lung[18]. In contrast, exposure of the lung to warm ischemia during implantation – when the lung is deflated and the organ slowly rewarms – has received remarkably little attention.

In liver and kidney transplantation however, it has been shown that the time to create the vascular anastomoses is an independent risk factor for short- and long-term outcome[19]. Prolonged anastomosis time (AT) in liver transplantation is associated with increased risk of early allograft dysfunction and graft loss in the early post-transplant period (<3 months)[20,21]. In kidney transplantation, AT is correlated with delayed graft function and long-term graft failure[22,23].

Based on these findings, it was our aim to investigate whether AT during double lung transplantation (DLTx) is an independent risk factor for development of PGD grade 3 (PGD3) within 72 hours post-transplant.

2. METHODS

2.1 Study design

This retrospective single-center cohort analysis included all DLTx patients at the University Hospitals of Leuven, Belgium between January 1st, 2008 until December 31st, 2016 with follow-up until September 5th, 2018. Retransplant patients, ex-vivo lung perfusion (EVLP) cases and patients who underwent single, lobar, redo or combined organ transplantation were excluded (**figure 1**).

Data were collected from written and electronical patient files, as well as donor data prospectively collected by Eurotransplant. Time between start and completion of data collection was prolonged due to the fact that a new database according the GDPR regulations had to be installed. The study was approved by the research ethics committee UZ/KU Leuven (MP008310).

2.2 Study population

Donor and recipient demographics as well as surgical and post-operative characteristics were selected based on potential correlations in prior studies with transplant outcome. In addition, possible confounders for developing PGD in relation to AT were considered.

Donor-related variables included age (years)[24,25], body mass index (BMI) in kg/m^2 [26], gender (male (M)/female (F))[25] and type (donation after brain death (DBD) *vs* DCD))[5]. Smoking history was not included due to 53 missing values, rendering a more complex statistical model (requiring multiple imputation) and reducing the strength of the analysis.

Recipient-specific variables included age[27], gender[28], BMI[28,29], indication for LTx[28] (emphysema, pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension (PAH) or other), previous thoracic surgery (pleural (chest-tube/pleurodesis), thoracic (open/thoracoscopic) or cardiac surgery)[30], pre-operative intensive care unit (ICU) stay, mechanical ventilation, ECLS pre-transplant[31] and pre-operative invasively measured mean pulmonary artery pressure (mPAP)[12] .

Surgical characteristics included cold ischemic time (CIT)[14], type of incision (bilateral thoracotomy/clamshell), intra-operative ECLS time[31], total blood product requirements (fresh frozen plasma, packed red blood cells and blood platelets)[32,33,34] and AT. CIT was defined as the interval between start of cold flush in the donor until the lung was removed from cold storage and positioned in the thoracic cavity of the recipient for implantation. In contrast to the blood products, we were unable to identify the per-operative total fluid resuscitation retrospectively from the individual files in an accurate and reliable way.

2.3 Outcome

Transplant outcomes included PGD, graft survival and overall patient survival. Uni- and multivariable analysis was performed to identify if AT is an independent risk factor for the primary outcome, defined as PGD3 within the first 72 hours post-transplant.

PGD was based on the International Society for Heart and Lung Transplantation (ISHLT) consensus and was assessed by pulmonary edema on X-ray and partial oxygen pressure divided by the fraction of inspired oxygen in arterial blood gases (PaO_2/FiO_2) at 0, 24, 48 and 72 hours post-transplant[11]. Grade 3 was assigned when the X-ray revealed pulmonary edema with a PaO_2/FiO_2 ratio <200 or when the combination of ECLS with bilateral pulmonary edema on X-ray occurred. Data on blood gases were acquired by an automated extraction of electronical patient files. X-rays were evaluated retrospectively by 2 experienced physicians, blinded for AT and outcome. In case of disagreement, consensus was reached.

2.4 Anastomosis time

AT was defined as the time interval between the end of the ice-cold preservation period and the restoration of blood flow to the lung graft, identified as the removal of the vascular clamps. We have no specific protocol for gradual opening of the clamps, which is performed immediately after implantation and takes 2-3 minutes. To analyze bilateral sequential lung implantation, the most relevant AT per patient was defined as the longest time interval when comparing implantation of the left with the right lung. Which lung was transplanted first depended on medical history, ventilation/perfusion scintigraphy, imaging or per-operative findings.

At our center, the lung graft is positioned in the thoracic cavity during implantation without any specific draping or topical cooling jacket. Running sutures were used for the (1) bronchial anastomosis (PDS 4/0), (2) pulmonary artery (Prolene 5/0) and (3) left atrial cuff (Prolene 4/0) starting in one corner on the back side and from both corners to the middle on the front side. Bronchial artery revascularization was not performed.

2.5 Statistical methods

Statistical analyses were performed using SAS9.4 (Windows) by an experienced biostatistician (SF). Continuous variables were reported as median (interquartile range (IQR)), categorical variables as number (percentage). Kaplan-Meier estimates visualize patient and graft survival (defined as graft loss or death without preceding graft loss).

Spearman correlations (ρ), Mann-Whitney U and Kruskal-Wallis tests were used to evaluate relations between covariates and AT. Logistic regression models were used for relations between AT and PGD3 within the first 72 hours post-LTx. Uni- and multivariable models were fitted. Before entering all variables in the regression model, multi-collinearity has been verified using the variance inflation factor. Without the categorical variable indication (having 5 levels) all variance inflation factors were lower than 2.6, hence indicating that multi-collinearity was not a problem.
Two alternative approaches were considered in the multivariable models: *no-model reduction strategy* (with all predefined confounders) and *backward model selection strategy*, with p=0.157 as critical value to stay in the model. The assumption of linearity in the regression models was verified by adding a quadratic term. P-values < 0.05 were considered statistically significant.

In an additional sub-analysis with an extension of the multivariable model, it was verified if the effect of AT on PGD3 differed between type of donor (DBD *vs* DCD) by inclusion of an interaction term.

3. RESULTS

3.1 Study population

A total of 551 LTx procedures were performed at the University Hospitals Leuven between January 1st, 2008, and December 31st, 2016 of which 510 were first DLTx and 41 were single-, lobar or multi-organ LTx. Twenty-six (4.7%) of the DLTx procedures were re-transplantations and were excluded as well as one patient who died at start of induction. Twenty-four EVLP cases were excluded for which a descriptive sub-analysis is summarized in supplementary **table S1 and S2**. This resulted in 459 DLTx of which data on AT and PGD3 was missing in 32 cases. The remaining 427 patients were included in our analysis (**figure 1**). The median follow-up was 5 years (2.7-7).

3.2 Recipient and donor demographics

Recipient and donor demographics are summarized in **table 1**. Overall, our study cohort had a median age of 56 years (47-60), of which 233 (50.8%) were male and 226 (49.2%) female. The most common indication for transplantation was emphysema, accounting for 271 cases (59%), followed by pulmonary fibrosis (n=92 (20%)) and cystic fibrosis (n=70 (15%)). Recipient BMI was 21.3kg/m^2 (18.7-25.3) and mPAP 37mmHg (31-44). Ninety-two patients (20%) underwent previous thoracic surgery. Fifteen patients (3.3%) had a pre-operative ICU stay, including 10 with ECLS as bridge to transplant. Bilateral thoracotomy was performed in 396 cases (86%) and clamshell in 63 (14%). Eighty-six (18.7%) patients required ECLS during transplantation, with a median intra-operative ECLS duration of 228 minutes (165-346) and median required blood products was 2 units (0-7).

Donors were aged 50 years (40-59), with a BMI of 24.5 kg/m^2 (22.5-27.0). The donor population comprised 240 (52.3%) male and 219 (47.7%) female donors of which 87 (19%) were DCD. Median CIT was 357 minutes (309-428). Median AT was 72 minutes (63-82). The distribution of AT, per 10-minute time intervals, is illustrated in **figure 2**.

One hundred thirty DLTx recipients (30.2% (out of 431 patients with available data on PGD)) developed PGD3 in the first 72 hours and 57 (13.2%) had PGD3 at 72 hours. Transplant outcomes are summarized in **table 2**. ICU stay was 6 days (4-12), of which 10 patients (2%) required post-operative ECLS for 3 days (3-7). The total length of in-hospital stay was 28 days (19-38). One, 3-

and 5-year patient survival was 94%, 87% and 82% respectively, and graft survival was 94%, 86% and 80%, respectively.

3.3 Potential confounding factors for prolonged AT

Relations between potential confounders and AT were explored (**table 3 and 4**). A positive correlation was observed between AT and increasing age of both recipient and donor (ρ=0.19, p<0.0001 and ρ=0.10, p=0.0459), as well as increasing BMI of the recipient and donor (ρ=0.23, p<0.0001 and ρ=0.15, p=0.0017) and total intra-operative blood product requirements (ρ=0.12, p=0.0115). AT was not associated with mPAP (ρ=0.07, p=0.1296).

Shorter AT was observed when access to the lung was reached via clamshell (65 minutes (59-79)) compared to bilateral anterior thoracotomy (73 minutes (64-83)) (p=0.018). Indication for transplant was not associated with the length of AT (p=0.051).

3.4 AT is an independent risk factor for development of PGD grade 3

3.4.1 Univariable analysis

Univariable analysis revealed a statistically significant positive correlation between AT and any PGD3 within 72h (OR per 10 min 1.293, 95%CI (1.136-1.471), p<0.0001) (**table 5**). The assumption of linearity was plausible, there was no evidence for a non-linear relation (quadratic term, p=0.1928). The probability for PGD3 was associated with an increasing length of AT, as illustrated in **figure 3A**.

Other correlations with PGD3 were observed for recipient BMI (OR 1.160, 95%CI (1.103-1.220), p<0.0001), pulmonary fibrosis (OR 2.530, 95%CI (1.523-4.203), p=0.0003), intra-operative ECLS (OR 1.003, 95%CI (1.001-1.005), p=0.0003), clamshell (OR 2.230, 95%CI (1.283-3.875), p=0.0045), CIT per 10 min (OR 1.046, 95%CI (1.021-1.071), p=0.0002) and total intra-operative blood products (OR 1.039, 95%CI (1.016-1.063), p=0.0008).

3.4.2 Multivariable analysis

In the multivariable logistic regression model, using the no-model reduction strategy, the positive linear relation between AT and any PGD3 within 72h remained (OR 1.205, 95%CI (1.022-1.421), p=0.0267) (**table 5**). There was no evidence for non-linearity (quadratic term p=0.1969). Other

variables that proved to be independent risk factors for developing PGD3 were recipient age (OR 0.957, 95%CI (0.925-0.989), p=0.0098) and BMI (OR 1.174, 95%CI (1.102-1.252), p<0.0001).

A similar association between AT and PGD3 was confirmed when a statistical model with backward selection was used (OR 1.222, 95%CI (1.063-1.405), p=0.0049). Also, recipient age, recipient BMI (OR 0.973, p=0.0064 and OR 1.177, p<0.0001) and intra-operative time on ECLS (OR 1.003, p=0.0081) revealed the same results as for the no-model building strategy (**table S3**).

A revision of the uni- and multivariable analysis was performed with the mean AT of both lungs. The positive linear relation between AT and PGD3 remained in the univariable setting (OR 1.401, 95%CI (1.189-1.650), p<0.0001). As well as in the multivariable setting, longer AT remained an independent factor associated with PGD3 (OR 1.270, 95%CI (1.028-1.569), p=0.0268) (**table S4**).

In the extension of the multivariable model (addition of an interaction term of AT with type of donor) there was no indication that the relation between AT and PGD3 differed between DCD and DBD lungs (p=0.83).

3.4.3 Effect on PGD grade 3 at 72 hours

In univariable analysis, AT was associated with an increased risk of development of PGD3 at 72h (OR per 10 min 1.294, 95%CI (1.1113-1.503), p=0.0008) (**figure 3B**), together with BMI of the recipient (OR 1.116, 95%CI (1.049-1.188), p=0.0005), intra-operative time on ECLS (OR 1.004, 95%CI (1.002-1.006), p<0.0001) and total intra-operative blood products (OR 1.046, 95%CI (1.021-1.071), p=0.0003). Multivariable logistic regression revealed AT as the strongest independent risk factor associated with PGD3 at 72h (OR per 10 min 1.267, 95%CI (1.044-1.539), p=0.0168). Other independent factors associated with PGD3 at 72h were BMI of the recipient and intra-operative time on ECLS (**table 6**). There was no significant association between mPAP and PGD3 at 72h.

When using mean AT, longer AT persisted to be the strongest independent risk factor for development of PGD3 at 72h (OR 1.467, p=0.0038) (**table S5**).

4. DISCUSSION

In this retrospective cohort study, we have shown for the first time, to our knowledge, that AT during DLTx is an independent risk factor for developing PGD3 within and at 72 hours post-transplant. The detrimental effect of a longer AT was similar in DCD versus DBD donors.

Previous studies have revealed several other correlations between surgical intervals during LTx and outcome. Kuntz et al.[13] performed a registry analysis of 6984 LTx between 1994 and 2002 and identified increased total ischemic time (time interval between aortic cross-clamp in the donor and reperfusion of the graft in the recipient) as independent risk factor for PGD. Using data from the INSPIRE trial (prospective study comparing cold storage with normothermic EVLP), ischemic time >300 minutes was demonstrated to have a strong correlation with PGD3[16]. With the increasing numbers of LTx from DCD donors, the focus on ischemic times has shifted from CIT to the donor agonal phase and DWIT. Levvey et al. did not find an adverse effect of donor agonal phase or DWIT up to 60 minutes on early survival, demonstrating the tolerability of the lung to these longer warm ischemic periods[17]. In contrast, exposure of the lung graft to warm ischemia during implantation (rewarming phase) has received little interest so far.

Our finding that the duration of AT negatively affects short-term outcome after LTx is consistent with recent studies in kidney and liver transplantation[19]. Heylen et al. retrospectively investigated the effect of AT in 669 kidney transplants from DBD donors[22] and revealed AT as an independent risk factor for decreased allograft function, reflected by an increased need for dialysis in the first 7 days post-transplant (OR 1.05, 95%CI (1.02–1.07), p=0.001).
In a multicenter retrospective study population of 13.964 kidney transplants, kidneys donated from DCD donors were less tolerant for longer AT compared to kidneys from DBD donors in regard to 5-year graft survival[23]. The researchers explained this by the additional detrimental effect of the agonal phase and DWIT, characteristic for DCD, resulting in a longer total WIT. In our study, the relation between AT and PGD3 did not differ between DCD and DBD lungs, indicating that DCD lungs, in comparison to kidneys, were more resistant to the detrimental impact of the rewarming phase during implantation. This is not surprising, since studies revealed no difference in outcome after LTx between DBD and DCD donors[17,35,36]. It is hypothesized that the oxygen reserve capacity of inflated lungs during the donor agonal phase protects the lungs from warm ischemia.

However, at the moment of implantation, the lung is deflated and therefore probably more susceptible to (re)warm(ing) ischemia.

The impact of AT on liver transplant outcome was studied in a retrospective Eurotransplant cohort study in 5223 recipients[20] and identified AT as an independent risk factor for graft loss within 3 months post-transplant (HR 1.08, 95%CI (1.05-1.12), p<0.001). Furthermore, it was observed that the effect of every 10-minute increase in AT was analog to the effect of each hour of additional CIT. A recent single-center study included 917 liver transplantations and showed an independent association between AT (for both portal vein and hepatic artery) and early allograft dysfunction[21]. Both liver transplant studies observed no evidence of a difference in regard to the magnitude of the effect of the AT between DCD and DBD donors which might be related to the low number of DCDs in the studies.

Based on our data, our hypothesis is that prolonged AT (and associated slowly rewarming ischemia) of a deflated lung graft in the thoracic cavity, enhances the detrimental effect of the inflammatory cascade of IRI[37], contributing to severe hypoxemia and lung edema (PGD).

These findings may contribute to new strategies to improve LTx outcome. Recognizing the existence of AT as independent risk factor might be sufficient for transplant surgeons to accelerate suturing time. However, in several cases, prolonged AT is unavoidable.
From an educational point of view, our findings might impact the way residents and fellows are trained for LTx. In our experience, we try to teach the technique gradually by starting with a well-exposed pulmonary vein, building up by adding the pulmonary artery and finally the bronchus. However, with the current findings, we suggest to include extensive training on large animal or phantom models in order to avoid prolonged suturing times. Time monitoring and suturing technique evaluation could also be considered.

Our results also revealed that clamshell incision results in a shorter AT compared to bilateral anterior thoracotomy. This could be attributed to the better exposure of the hilum and the improved working space. However, the adverse effects of this incision (potential wound problems, sacrifice of the mammary arteries, sternal dehiscence and compression of the inferior sternum on the right ventricle throughout the procedure) should also be taken into account.

Furthermore, techniques to prevent the lung from rewarming during implantation could be considered. In 1993, Date et al. evaluated a pulmonary cooling jacket in a canine model of LTx, which resulted in uniform cooling of the lung graft during implantation. Significant increase in temperature was observed in its absence, despite topical cooling by cold saline solution[38]. In our clinical practice, no cooling jackets are used. It is important to recognize that the use of a cooling jacket can render the implantation more difficult due to lack of space (especially in case of a small chest cavity as in pulmonary fibrosis). On the other hand, topical cooling with the application of slushed ice directly on the lung graft may potentially injure the phrenic nerves[39]. The effect of topical cooling during implantation was studied in a pig model of LTx in comparison to a group of slow rewarming. Unfortunately, the temperature of the lung was not measured. No difference in pulmonary edema post-transplant was observed. It was even mentioned that a tendency was seen towards lower vascular resistance in the slowly rewarming group, due to less vasoconstriction[40]. A recent study showed that optimal reperfusion conditions can be reached by the use of elective intra-operative ECLS, resulting in lower PGD incidences[41].

To better understand the process of rewarming during implantation and detect strategies to overcome its detrimental effect on PGD, it is needed to objectify the core temperature of the lung during implantation with a central probe. We also intent to validate our results in a multicenter cohort and compare centers with different techniques of implantation. As a result of our current study findings, we have now started to keep the donor lung cool during implantation, by intermittent topical administration of cold preservation solution.

Our current study has some inherent limitations due to its single-center and retrospective character. Although we considered several potential confounding factors, we may have overlooked residual confounders and other known risk factors (e.g. mPAP) were not confirmed in our series. As PGD is scored on X-ray and blood gases of both lungs, it is worth mentioning that we have revised the analysis with the mean AT of both lungs instead of maximum AT of a single lung, resulting in the same findings.

In conclusion, AT is identified as an independent risk factor for the development of PGD3 in the initial 72 hours post-transplant. Further research is warranted to better understand the rewarming phase during implantation and investigate methods to prevent its deleterious effect.

MANUSCRIPT SUMMARY

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Disclosure

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Data availability statement:

The data that support the findings of this study are available on request from the corresponding author. All authors contributed in an important manner to the study design, data collection and analysis, or writing of the paper according to the guidelines of the International Committee of

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  - Contributed important ideas, collected data, critically revised the data and the manuscript
- Cedric Vanluyten (BSc)
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- Steffen Fieuws (PhD)
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- Ina Jochmans (MD, PhD)
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- Bart M. Van Audenaerde (PhD)
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- Laurent Godinas (MD, PhD)
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- Geert M. Verleden (MD, PhD)
    - Contributed important ideas, critically revised the manuscript
- Dirk E. Van Raemdonck (MD, PhD)
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- Laurens J. Ceulemans (MD, PhD)
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FIGURE LEGENDS

Figure 1: Flowchart diagram of the study cohort.

Patients who underwent lobar, single lung, multi-organ or re-transplantation were excluded as well as one patient who died during induction (n=68). Ex-vivo lung perfusion cases (n=24) were excluded. Of the remaining 459 patients, anastomosis time (AT) was missing in 4 cases and any Primary Graft Dysfunction grade 3 (PGD3) in 24 cases, both parameters (AT and PGD3) were missing in 4 patients. 427 of all the lung transplant procedures performed during the study period are included in our analysis.

AT, anastomosis time; DLTx, double lung transplantation; EVLP, ex-vivo lung perfusion; LTx, lung transplantation; PGD3, primary graft dysfunction grade 3.

Figure 2: Distribution of anastomosis time (maximum left/right) per 10-minute time intervals.

Figure 3: Univariable logistic regression of the probability of development of (A) any PGD grade 3 within 72 hours, and (B) PGD grade 3 at 72 hours with increasing anastomosis time.

The shaded area represents the 95% confidence interval. PGD, primary graft dysfunction.

TABLE LEGENDS

Table 1: Overview of recipient and donor demographics.

n = 459. Data are expressed as median (IQR) if not otherwise indicated.

DBD, donation after brain death; DCD, donation after circulatory death; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; min, minutes.

Table 2: Overview of post-operative outcomes after double lung transplantation.

Data are expressed as median (IQR) if not otherwise indicated.

[+]Percentage is calculated based on the available data on PGD3 (n=431).

[++]Graft loss, or death without preceding graft loss.

CI, confidence interval; ECLS, extracorporeal life support; ICU, intensive care unit; PGD, primary graft dysfunction.

Table 3: Continuous variables associated with anastomosis time.

Overview Spearman correlations with maximal (left/right) anastomosis time.

[+] Size mismatch in absolute difference in centimeters.

BMI, body mass index; CI, confidence interval; ECLS, extracorporeal life support; min, minutes.

Table 4: Categorical variables associated with anastomosis time.

Variables are analyzed using a Mann-Whitney U test or a Kruskal-Wallis test. All reported p-values are two-sided. Data are expressed as median (IQR). Maximal anastomosis time (left/right).

AT, anastomosis time; DBD, donation after brain death; DCD, donation after circulatory death; ECLS, extracorporeal life support; ICU, intensive care unit; PAH, pulmonary arterial hypertension.

Table 5: Univariable and multivariable logistic regression for any PGD grade 3 within 72 hours.

[a]Results from univariable logistic regression models. AUC=area under the operating characteristics curve or C-index (index of discrimination): 0.5=random prediction, 1=perfect discrimination. For all continuous predictors, a linear relation (on the logit scale) was plausible (this has been verified using restricted cubic splines).

[b]Results from a multivariable logistic regression model based on 129 events from 427 subjects, using no model building strategy.

reference category; [+]Size mismatch in absolute difference in centimeters.

BMI, body mass index; DBD, donation after brain death; DCD donation after circulatory death; ECLS, extracorporeal life support; ICU, intensive care unit; OR, odds ratio; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction.

Table 6: Univariable and multivariable logistic regression for PGD grade 3 at 72 hours.

[a]Results from univariable logistic regression models. AUC=area under the operating characteristics curve or C-index (index of discrimination): 0.5=random prediction, 1=perfect discrimination. For all continuous predictors, a linear relation (on the logit scale) was plausible (this has been verified using restricted cubic splines).

[b]Results from a multivariable logistic regression model based on 57 events from 431 subjects, using no model building strategy.

reference category; [+]Size mismatch in absolute difference in centimeters.

BMI, body mass index; DBD, donation after brain death; DCD donation after circulatory death; ECLS, extracorporeal life support; ICU, intensive care unit; OR, odds ratio; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction.

SUPPORTING INFORMATION STATEMENT

Additional supporting information may be found online in the Supporting Information section.

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FIGURES

Figure 1: Flowchart diagram of the study cohort.

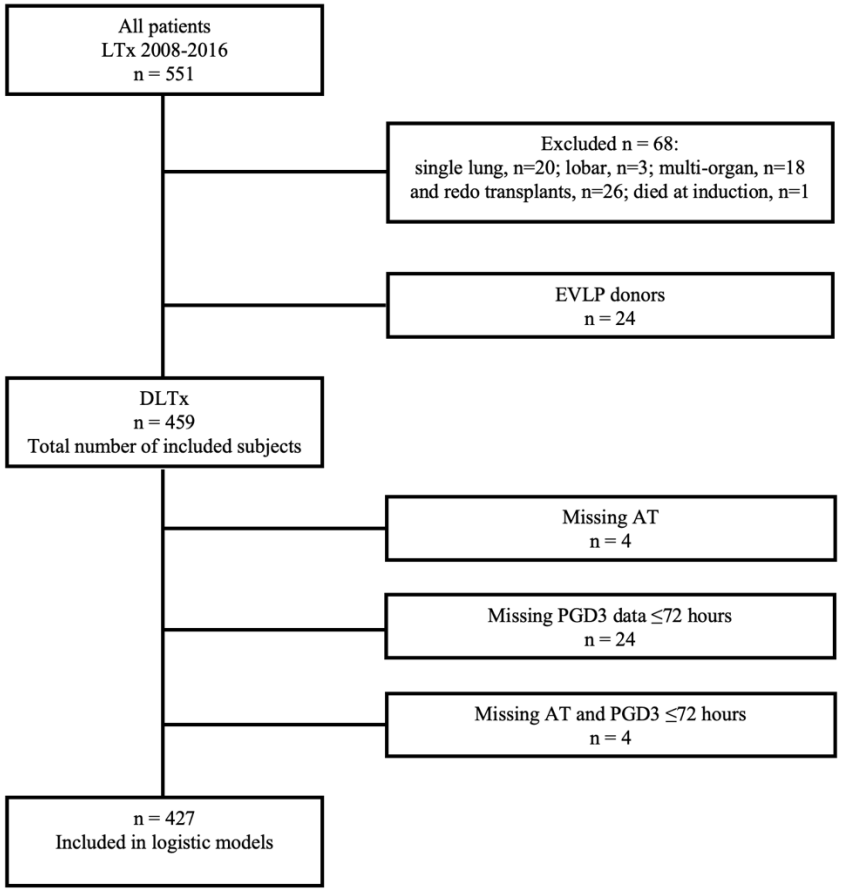


Figure 2: Distribution of anastomosis time (maximum left/right) per 10-minute time intervals.

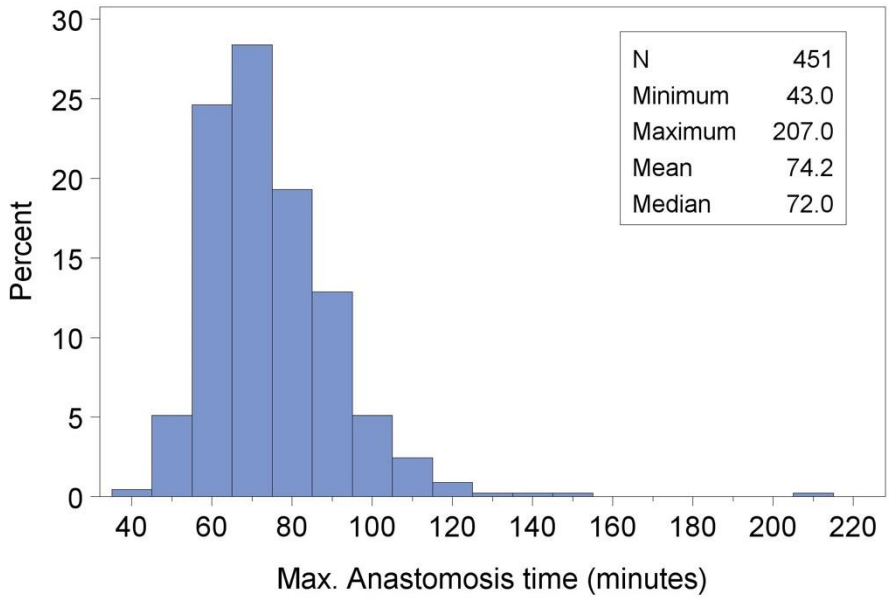
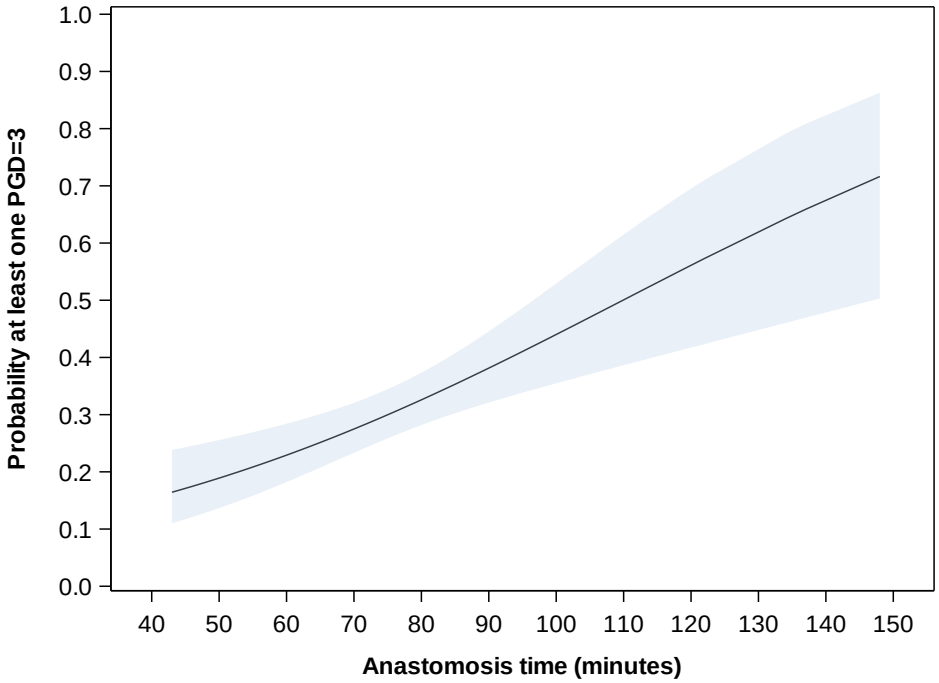
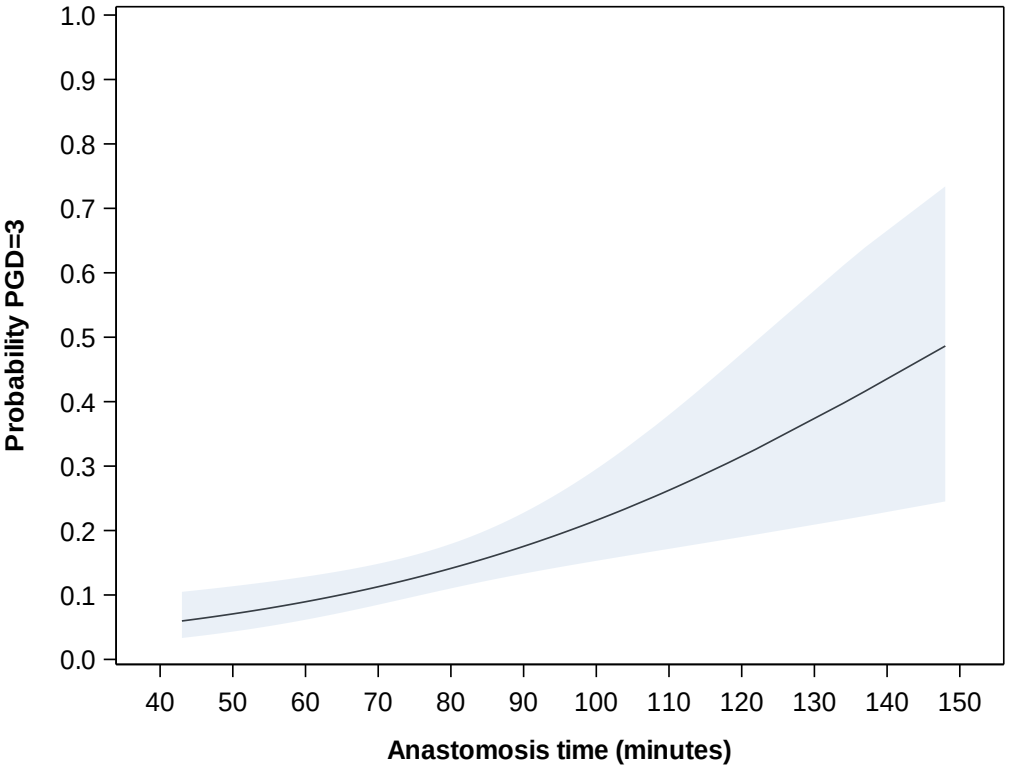


Figure 3: Univariable logistic regression of the probability of development of

(A) any PGD grade 3 ≤72 hours with increasing anastomosis time.



(B) PGD grade 3 at 72 hours with increasing anastomosis time.



TABLES

Table 1: Overview of recipient and donor demographics.

Characteristics	**Results**
Recipient	
Age at transplant, years	56 (47-60)
Sex, n (%)	
Male	233 (50.8)
Female	226 (49.2)
Body mass index, kg/m^2	21.30 (18.69-25.32)
Indication for transplant, n (%)	
Emphysema	271 (59)
Pulmonary Fibrosis	92 (20)
Cystic Fibrosis	70 (15)
Pulmonary Arterial Hypertension	13 (3)
Other disorders	13 (3)
Pre-operative ICU stay, n (%)	15 (3.3)
Pre-operative ECLS, n (%)	10 (2.2)
Pre-operative thoracic surgery, n (%)	92 (20)
Donor	
Type donor, n (%)	
DBD	372 (81)
DCD	87 (19)
Age at donation, years	50 (40-59)
Sex, n (%)	
Male	240 (52.3)
Female	219 (47.7)
Body mass index, kg/m^2	24.46 (22.49-27.04)
Surgical	
Type of incision, n (%)	
Bilateral thoracotomy	396 (86.3)
Clamshell	63 (13.7)

Intra-operative ECLS, n (%)	86 (18.7)
Cardiopulmonary bypass, n (%)	3 (3.5)
ECMO, n (%)	83 (96.5)
Intra-operative ECLS time, min	228 (165-346)
Cold ischemic time (longest time of 2 lungs), min	357 (309-428)
Cold ischemic time (mean of 2 lungs), min	279 (241-335)
Anastomosis time (longest time of 2 lungs), min	72 (63-82)
Anastomosis time (mean of 2 lungs), min	67 (60-74)
Mean pulmonary artery pressure, mmHg	37 (31-44)
Total intra-operative blood products, units	2 (0-7)

Legend: n = 459. Data are expressed as median (IQR) if not otherwise indicated.
DBD, donation after brain death; DCD, donation after circulatory death; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; min, minutes.

Table 2: Overview of post-operative outcomes after double lung transplantation.

Outcomes	Results
Any PGD grade 3 ≤72 hours, n (%)[+]	130 (30.2)
PGD grade 3 at 72 hours, n (%)[+]	57 (13.2)
Post-operative ECLS, n (%)	10 (2)
Time on post-operative ECLS, days	3 (3-7)
ICU length of stay, days	6 (4-12)
Total hospital length of stay, days	28 (19-38)
Survival	
Overall patient survival, years since transplant, % (95% CI)	
1 year	94 (92-96)
3 years	87 (83-89)
5 years	82 (78-85)
Graft survival[++], years since transplant, % (95% CI)	
1 year	94 (91-96)
3 years	86 (83-89)
5 years	80 (75-82)

Legend: Data are expressed as median (IQR) if not otherwise indicated.

[+]Percentage is calculated based on the available data on PGD3 (n=431).

[++]Graft loss, or death without preceding graft loss.

CI, confidence interval; ECLS, extracorporeal life support; ICU, intensive care unit; PGD, primary graft dysfunction.

Table 3: Continuous variables associated with anastomosis time.

Variables	**Spearman ρ**	**P value**
Age recipient, years	0.189	**<.0001**
BMI recipient, kg/m^2	0.226	**<.0001**
Age donor, years	0.094	**0.0459**
BMI donor, kg/m^2	0.147	**0.0017**
Size mismatch$^+$	-0.008	0.8735
Cold ischemic time (max), min	0.378	**<.0001**
Cold ischemic time (mean), min	0.285	**<.0001**
Intra-operative ECLS time, min	0.057	0.2232
Mean pulmonary artery pressure, mmHg	0.071	0.1296
Total intra-operative blood products, units	0.119	**0.0115**

Legend: Overview Spearman correlations with maximal (left/right) anastomosis time.

$^+$ Size mismatch in absolute difference in centimeters.

BMI, body mass index; CI, confidence interval; ECLS, extracorporeal life support; min, minutes.

Table 4: Categorical variables associated with anastomosis time.

Variables	**AT (minutes)**	**P value**
Type of incision		**0.018**
Bilateral thoracotomy	73 (64-83)	
Clamshell	65 (59-79)	
Pre-operative ICU stay		0.803
Yes	72 (64-90)	
No	72 (63-82)	
Type of donor		0.952
DCD	71 (62-82)	
DBD	73 (63-82)	
Pre-operative ECLS		0.115
Yes	79 (70-98)	
No	72 (63-82)	
Previous thoracic surgery		0.195
Yes	73 (64-87)	
No	72 (62-81)	
Indication for transplantation		0.051
Cystic Fibrosis	69 (62-77)	
Emphysema	72 (62-83)	
PAH	77 (62-89)	
Pulmonary Fibrosis	75 (67-87)	
Other disorders	66 (63-73)	

Legend: Variables are analyzed using a Mann-Whitney U test or a Kruskal-Wallis test. All reported p-values are two-sided. Data are expressed as median (IQR). Maximal anastomosis time (left/right).

AT, anastomosis time; DBD, donation after brain death; DCD, donation after circulatory death; ECLS, extracorporeal life support; ICU, intensive care unit; PAH, pulmonary arterial hypertension.

Table 5: Univariable and multivariable logistic regression for any PGD grade 3 ≤ 72 hours.

	Univariable logistic regression[a]		Multivariable logistic regression[b]	
Variables	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**
Anastomosis time (max), per 10 min	**1.293 (1.136-1.471)**	**<.0001**	**1.205 (1.022-1.421)**	**0.0267**
Type donor				
DCD	1.019 (0.604-1.719)	0.9430	0.957 (0.707-1.296	0.7765
DBD	#	.	#	.
Age recipient, year	0.995 (0.979-1.011)	0.5493	**0.957 (0.925-0.989)**	**0.0098**
BMI recipient, kg/m^2	**1.160 (1.103-1.220)**	**<.0001**	**1.174 (1.102-1.252)**	**<.0001**
Indication		**0.0038**		0.4045
Cystic Fibrosis	0.950 (0.506-1.783)	0.8730	0.883 (0.389-2.001)	0.7647
PAH	2.494 (0.809-7.687)	0.1116	0.964 (0.291-3.193)	0.9522
Pulmonary Fibrosis	**2.530 (1.523-4.203)**	**0.0003**	2.035 (0.995-4.163)	0.0516
Rare disorders	0.831 (0.169-4.102)	0.8207	0.339 (0.068-1.704)	0.1892
Emphysema	#	.	#	.
Pre-operative ICU stay		.		.
Yes	1.465 (0.470-4.566)	0.5102	1.097 (0.547-2.200)	0.7952
No	#	.	#	.
Pre-operative ECLS		.		
Yes	1.161 (0.286-4.717)	0.8342	0.471 (0.175-1.268)	0.1365
No	#	.	#	.
Intra-operative ECLS, min	**1.003 (1.001-1.005)**	**0.0003**	1.001 (0.998-1.004)	0.4124
Pre-operative thoracic surgery		.		.
Yes	1.543 (0.927-2.569)	0.0956	1.135 (0.832-1.550)	0.4239
No	#	.	#	.
Type of incision		.		.
Clamshell	**2.230 (1.283-3.875)**	**0.0045**	1.164 (0.798-1.698)	0.4312
Bilateral thoracotomy	#	.	#	.
Age donor, year	1.000 (0.986-1.014)	0.9963	1.009 (0.991-1.027)	0.3428
BMI donor, kg/m^2	0.987 (0.938-1.039)	0.6174	0.971 (0.913-1.033)	0.3460
Size mismatch^{++}	0.993 (0.953-1.034)	0.7255	1.005 (0.959-1.052)	0.8478
Cold ischemic time (max), per 10 min	**1.046 (1.021-1.071)**	**0.0002**	1.011 (0.980-1.043)	0.4940
Mean pulmonary artery pressure, mmHg	1.002 (0.988-1.017)	0.7502	0.985 (0.967-1.003)	0.1097
Total intra-operative blood products, units	**1.039 (1.016-1.063)**	**0.0008**	1.017 (0.979-1.056)	0.3965

Legend: [a]Results from univariable logistic regression models. AUC=area under the operating characteristics curve or C-index (index of discrimination): 0.5=random prediction, 1=perfect discrimination. For all continuous predictors, a linear relation (on the logit scale) was plausible (this has been verified using restricted cubic splines).

[b]Results from a multivariable logistic regression model based on 129 events from 427 subjects, using no model building strategy.

reference category; [+]Size mismatch in absolute difference in centimeters.

BMI, body mass index; DBD, donation after brain death; DCD donation after circulatory death; ECLS, extracorporeal life support; ICU, intensive care unit; OR, odds ratio; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction.

Table 6: Univariable and multivariable logistic regression for PGD grade 3 at 72 hours.

	Univariable logistic regression[a]		Multivariable logistic regression[b]	
Variables	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**
Anastomosis time (max), per 10 min	**1.294 (1.113-1.503)**	**0.0008**	**1.267 (1.044-1.539)**	**0.0168**
Type donor				
DCD	1.267 (0.649-2.475)	0.4884	1.126 (0.761-1.666)	0.5540
DBD	#	.	#	.
Age recipient, year	0.988 (0.968-1.008)	0.2201	0.967 (0.929-1.008)	0.1117
BMI recipient, kg/m^2	**1.116 (1.049-1.188)**	**0.0005**	**1.116 (1.028-1.212)**	**0.0086**
Indication		0.0735		0.9761
Cystic Fibrosis	1.453 (0.645-3.272)	0.3675	1.064 (0.410-2.758)	0.8985
PAH	**4.018 (1.156-13.959)**	**0.0286**	0.866 (0.244-3.082)	0.8246
Pulmonary Fibrosis	**2.260 (1.158-4.408)**	**0.0168**	1.205 (0.538-2.699)	0.6509
Rare disorders	2.009 (0.412-9.799)	0.3884	0.971 (0.186-5.066)	0.9718
Emphysema	#	.	#	.
Pre-operative ICU stay		.		.
Yes	2.002 (0.534-7.501)	0.3031	1.290 (0.571-2.916)	0.5405
No	#	.	#	.
Pre-operative ECLS		.		
Yes	1.888 (0.383-9.317)	0.4353	0.451 (0.148-1.368)	0.1595
No	#	.	#	.
Intra-operative ECLS, min	**1.004 (1.002-1.006)**	**<.0001**	**1.003 (1.000-1.007)**	**0.0460**
Pre-operative thoracic surgery		.		.
Yes	1.313 (0.671-2.567)	0.4263	1.037 (0.687-1.565)	0.8636
No	#	.	#	.
Type of incision		.		.
Clamshell	**3.221 (1.684-6.160)**	**0.0004**	1.303 (0.848-2.000)	0.2267
Bilateral thoracotomy	#	.	#	.
Age donor, year	1.009 (0.990-1.028)	0.3694	1.024 (0.999-1.049)	0.0653
BMI donor, kg/m^2	0.949 (0.879-1.025)	0.1811	0.912 (0.830-1.001)	0.0532
Size mismatch^{++}	0.999 (0.947-1.054)	0.9682	1.012 (0.954-1.073)	0.6979
Cold ischemic time (max), per 10 min	**1.047 (1.017-1.077)**	**0.0018**	1.000 (0.961-1.041)	0.9979
Mean pulmonary artery pressure, mmHg	1.008 (0.989-1.027)	0.3983	0.987 (0.964-1.010)	0.2726
Total intra-operative blood products, units	**1.046 (1.021-1.071)**	**0.0003**	1.001 (0.959-1.046)	0.9531

Legend: [a]Results from univariable logistic regression models. AUC=area under the operating characteristics curve or C-index (index of discrimination): 0.5=random prediction, 1=perfect discrimination. For all continuous predictors, a linear relation (on the logit scale) was plausible (this has been verified using restricted cubic splines).

[b]Results from a multivariable logistic regression model based on 57 events from 431 subjects, using no model building strategy.

reference category; [+]Size mismatch in absolute difference in centimeters.

BMI, body mass index; DBD, donation after brain death; DCD donation after circulatory death; ECLS, extracorporeal life support; ICU, intensive care unit; OR, odds ratio; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction.