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Inflammation and primary graft dysfunction after lung transplantation: CT-PET findings

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Abstract

BACKGROUND: The leading cause of early mortality after lung transplantation is Primary Graft Dysfunction (PGD). We assessed the lung inflammation, inflation status and inhomogeneities after lung transplantation. Our purpose was to investigate the possible differences between patients who did or did not develop PGD.

METHODS: We designed a prospective observational study enrolling patients who underwent a (CT-PET) study within 1 week after lung transplantation. Twenty-four patients (10 after double- and 14 after single-lung) were enrolled. Respiratory and hemodynamic data were collected before, during and after lung transplantation. Each patient underwent Computed Tomography - Positron Emission Tomography (CT-PET) scan early after surgery. broncho-alveolar lavage (BAL) fluid collection was performed to analyze inflammatory mediators.

RESULTS: The grafts showed a $[^{18}F]$fluoro-2-deoxy-D-glucose ($[^{18}F]$FDG) uptake rate of 26[18-33]$*10^{-4}$ mL_{blood}/mL_{tissue}/min (reference values 11[7-15]$*10^{-4}$). Three double- and 6 single-lung recipients developed PGD. The grafts of patients who developed PGD had similar $[^{18}F]$FDG uptake than grafts of patients who did not (28[18-26]$*10^{-4}$ versus 26[22-31]$*10^{-4}$, p=0.79). Not-inflated tissue fraction was significantly higher (28[20-38]% versus 14[7-21]%, p=0.01) while well-inflated fraction was significantly lower (29[25-41]% versus 53[39-65]%, p<0.01). Inhomogeneity extent was higher in patients who developed PGD (23[18-26]% versus 14[10-20]%, p=0.01)The lung weight was 650[591-820]g versus 597[480-650]g (p=0.09)). BAL fluid analysis for inflammatory mediators did not detect a difference between the study groups. CONCLUSIONS: Compared to healthy lungs, all the grafts showed increased $[^{18}F]$FDG uptake rate, but there were no differences between patients who developed PGD and patients who did not. Of note, the PGD patients showed a worse inflation status of lungs and a higher inhomogeneity extent.

Key words: lung transplantation, Primary Graft Dysfunction, CT-PET, respiratory failure, hemodynamic impairment, VILI.

Introduction

Lung transplantation is the only effective therapy for end-stage lung disease[1]. Primary graft dysfunction (PGD) is the most immediate post-transplant complication and the leading cause of early mortality in these patients[2].

PGD is a clinical syndrome defined in 2005[3] to describe pulmonary edema occurring within the first 72 hours after lung transplantation. Diagnosis is based on reduced oxygenation (measured as partial pressure of oxygen in arterial blood/inspired fraction of oxygen ratio <300) with a chest X-ray showing bilateral infiltrates and no other readily identified secondary causes of graft dysfunction. The PGD is associated with worse functional outcome[4] and Bronchiolitis Obliterans Syndrome development, which complicates late period after lung transplantation reducing survival[5].

The mechanisms underlying the development of PGD are not completely understood, although most hypotheses point to an inflammatory origin of the edema. Ischemia-reperfusion injury seems to play a central role[6–8], though the inflammatory cascade could also result from surgical stress, manipulation of the lungs, and the matching of donor/recipient immunity cells[9].

However, there is still no direct quantification of the inflammatory activation and its relationship with PGD. Therefore, in this study we measured the rate of uptake of [18F]fluoro-2-deoxy-D-glucose ([18F]FDG) by positron emission tomography (PET)[10], as a possible marker of inflammation. In literature, a PET study was performed late in 15 recipients after lung transplantation in order to find an non invasive alternative technique to transbronchial biopsies able to discriminate between rejection and infection[11]. In the early phase after lung transplantation, we posed the following questions: 1) Is the rate of [18F]FDG uptake different in grafts of patients who developed PGD? 2) Does [18F]FDG uptake rate increase in transplanted lungs compared to normal values reported in healthy lungs? If so, to what extent? 3) In single-lung transplant patients, is there any difference in [18F]FDG uptake rate between the grafts and native not-transplanted lungs?

Materials and Methods

Patients

Patients who underwent single- or double-lung transplantation at IRCCS Fondazione Cà Granda – Ospedale Maggiore Policlinico (Milan, Italy) between 2013-2015 were enrolled. The study protocol was approved by the hospital ethical review board and the trial was registered with www.clinicaltrials.gov (NCT 01746914).

Data acquisition

Pre-operative, intra-operative and post-operative data

Pulmonary and hemodynamic variables were prospectively collected from surgery up to 72h at standardized time-points (see the Additional File for details). The CT-PET study was performed within 7 days after surgery. In our CT-PET facility we could perform the exam in patients intubated under mechanical ventilation or in patients who breathed spontaneously, also with oxygen supplementation. At least 4 hours before CT-PET study, a broncho-alveolar lavage (BAL) of the grafts was done and samples were collected for culture, cytometric and inflammatory mediators' analysis (bFGF, CXCL-8/IL-8, CXCL10/IP-10, CCL2/MCP-1, MMP7, RAGE, TIMP1, IL-6, IL-1β, TNF-α, SERPINE1/PAI-1; see the Additional File for detailed methods of inflammatory mediators' analysis).

CT-PET study

The CT-PET study (Biograph 64 TruePoint, Siemens, Germany®) started with a bolus of [^{18}F]FDG injected in 30s (300 MBq, range 260-390 MBq), followed by dynamic CT acquisition (low-dose scan protocol) and dynamic PET acquisition. Dynamic PET frames (24x5s, 6x180s, 7x300s) were acquired over 60 minutes. During PET imaging, blood samples were sequentially drawn at standardized time-points to measure [^{18}F]FDG blood content. Blood sample-activity concentrations were measured with a well counter (WIZARD, Perkin-Elmer) cross-calibrated with

the PET scanner[12].

PET images

PET images were reconstructed on 74 slices (matrix 128x128; voxel size 3x3x3mm). The graphic Patlak approach[13] was employed for dynamic PET volumes to estimate voxel-by-voxel [^{18}F]FDG uptake rates: after skipping PET frames taken during the first 8 minutes of acquisition, for each time-point (from 10 to 60 minutes), voxel-by-voxel we recorded lung activity and normalized it to blood activity. These values were plotted against the integral of plasma activity normalized to blood activity. The slope of the linear part of the graph represents [^{18}F]FDG voxel uptake ($mL_{blood}/mL_{tissue}/min$). This parametric volume was then registered to the resolution of CT scan (512x512) so as not to lose information[12].

CT analysis[12]

A dedicated software was used for CT analysis (www.softefilm.eu). After outlining the lung profiles by hand, gas and tissue volumes were determined. Each voxel was classified according to its CT number as not (HU >-100), poorly (-100>HU>-500), well- (-500>HU>-900) or over- (HU < -900) inflated[14].

Lung inhomogeneities were determined by measuring the gas/tissue ratio in two contiguous lung regions. We defined the "extent" of the inhomogeneities as the fraction of lung volume with inhomogeneities greater than 1.61, 95 percentile of normal population[15], as previously defined[16].

Definition of Primary Graft Dysfunction

Patients were classified in those who developed PGD and those who did not develop PGD. The clinical diagnostic criteria for PGD were[3]: 1) presence of infiltrates consistent with diffuse pulmonary edema at the chest X-ray and 2) progressive hypoxemia (PaO_2/FiO_2 <300). The chest X-

rays were independently evaluated by a radiologist and an intensivist. The severity of PGD is graded based on the PaO_2/FiO_2 ratio:

- Grade 1 – $PaO_2/FiO_2 > 300$

- Grade 2 – PaO_2/FiO_2 between 200 and 300

- Grade 3 – $PaO_2/FiO_2 < 200$

Exclusion criteria were: hyperacute rejection, venous anastomotic obstruction, cardiogenic pulmonary edema and pneumonia (both viral and bacterial).

Statistical Analysis

Sample size was calculated on the basis of two previously published studies. Normal $[^{18}F]$FDG uptake rate in 10 healthy subjects had been reported to be 10.9 ± 3.1 mL$_{blood}$/mL$_{tissue}$/min[10] while in Acute Respiratory Distress Syndrome (ARDS) patients average $[^{18}F]$FDG uptake rate was 56 ± 38 mL$_{blood}$/mL$_{tissue}$/min[12]. As the oxygenation impairment in ARDS definition[17] and PGD[3] are similar we hypothesized that, if inflammation was the main cause of the clinical impairment in patients who developed PGD Grade 2 and 3, also the $[^{18}F]$FDG uptake rate should be similar to ARDS patients. In order to find a statistically significant difference between patients who developed PGD (we assumed to present a $[^{18}F]$FDG uptake rate similar to ARDS patients) and patients who did not (we assumed to have a $[^{18}F]$FDG uptake rate similar to healthy subjects) with a statistical power of 80% and type 1 error of 5%, we need a sample size of almost 6 patients each group.

Continuous variables were compared with the Wilcoxon signed rank test, and categorical variables with Fisher's exact test or Chi Square test (if the 2x2 factors were more than 5). Data are shown as median [interquartile rage]. R software was used for statistical analysis[18].

Results

Twenty-four patients were enrolled; 14 underwent single-lung transplantation (14 grafts), and 10 double-lung transplantation (20 grafts) (see the Additional File, Table E1 and Table E2 for demographic and anthropometric characteristics of recipients and donors). CT-PET studies were performed after a median of 5 [4-6] days after transplantation, all during the first week after surgery, as described in the protocol of the study.

During the period between the surgery and the CT-PET study, only 1 patient developed a body temperature > 38°C (single event one morning); 10 patients developed bacterial colonization of the tracheobronchial tree. Moreover, 6 patients showed a wedge pressure > 18 mmHg, all in the first 24 hours after surgery; 4 of them normalized the wedge pressure value after the stabilization period of 6 hours in ICU.

[^{18}F]FDG uptake rate

Table 1 summarizes the CT scan characteristics and [^{18}F]FDG uptake rates of the 48 lungs analyzed (grafts and native not-transplanted lungs). On average [^{18}F]FDG uptake rate was increased compared to reference normal values[10] (27 ± 12 vs 10.9 ± 3.1, $p>0.001$) but lower than the one measured in ARDS patients[12] (27 ± 12 vs 56 ± 38, $p<0.001$). The [^{18}F]FDG uptake rate decreased with time from transplantation in the grafts, even if the correlation was weak ($R^2=0.28$, $p=0.001$) (see the Additional File, Figure E1). It was weakly related to lung inhomogeneity ($R^2=0.13$, $p=0.03$) (see the Additional File, Figure E2) and rose significantly along the sternum-vertebral axis (see Figure 1).

In patients who underwent double-lung transplantation we found no differences between the first and second grafts reperfused in recipients. In particular, the lung weight (p=0.63), the inflation status (not inflated tissue p=0.97, poorly inflated tissue p=0.63, well inflated tissue p=0.68, over inflated tissue p=0.91), the inhomogeneity extent (p=0.63) and the [^{18}F]FDG uptake rate (p=0.91) were similar in the two grafts groups. Moreover, we could not find any relationship between ischemia times (cold and warm ischemia) and [^{18}F]FDG uptake rates in our patients (see the

Additional File, Table E3).

Primary graft dysfunction

The PGD developed in 9 out of 24 patients (37.5%, 6 single-lung and 3 double-lung transplants). Table 2 compares the grafts which developed PGD and the grafts which did not (see the Additional File, Table E5). The [^{18}F]FDG uptake rates (*10^{-4} mL$_{blood}$/mL$_{tissue}$/min) were similar in grafts of patients who did or did not develop PGD (28 [18-26] versus 26 [22-31], p=0.79) (Figure 2).

The indices of systemic inflammation (leukocyte count and reactive C protein), broncho-alveolar lavage fluid examination and inflammatory mediators were similar in the two patients' groups, except for a trend toward an increase in TIMP1 in PGD patients (37[16 – 244] versus 14[6 – 28]ng/ml, p=0.06) (see the Additional File, Table E6, E7, E8). The incidence of bacterial colonization in broncho-alveolar lavage fluid was also similar in the two groups (OR 0.45, 95% C.I. 0.05-3.17, p=0.42). There was no difference in time between lung transplantation and CT-PET study between patients who did and did not develop PGD (4.5[4-5] versus 5[4-6] days, p=0.29).

PGD lungs presented a lower fraction of well inflated tissue and a higher fraction of not inflated tissue compared to patients without PGD. In addition patients who developed PGD showed a greater graft inhomogeneity and tended to have a higher lung weight than grafts of patients who did not (p=0.09). The slope of lung weight increase along the sternum-vertebral axis was significantly steeper in grafts of patients who developed PGD than in grafts of patients who did not (p=0.004, see the Additional File, Figure E8).

Hemodynamic and respiratory variables of patients who did or did not develop PGD are reported in the Additional File, Tables E10 and E11. The arterio-venous difference in oxygen content was significantly greater during surgery (3.4[3.3-3.8] versus 2.8 [2.2-3.2]ml/dl, p=0.01) and

the shunt fraction was significantly higher in the postoperative period (23.1[19.7-27.8] versus 13.2[11.2-17.5]% at arrival in Intensive Care Unit, p=0.02) in patients who developed PGD. Postoperatively, heart rate (94[86-102] versus 79[74-84]bpm at arrival in ICU, p=0.02), central venous pressure (13[9-14] versus 9[8-10]mmHg 6 hours after arrival in ICU, p=0.04) and fluid balance (722[11-2600] versus -757[-1230;1000]ml during the first 24 hours in ICU, p=0.04) were also significantly greater in patients who developed PGD.

Data about the outcome are reported in the Additional File (Table E9). In summary, data showed a worse respiratory outcome in patients who developed PGD.

Morphologic and metabolic features of the 14 grafts and 14 native not-transplanted lungs after single lung transplantation

The [^{18}F]FDG uptake rates were similar in the grafts and native not-transplanted lungs (p=0.19). As shown in Table 1, the lung weight of the grafts and the native not-transplanted lungs was similar (p=0.70). Single-lung transplant patients had significantly more poorly-inflated tissue in the native not-transplanted lungs than the grafts (p<0.01). In addition, inhomogeneity extent was significantly greater in the native not-transplanted lungs than in the grafts (p=0.01).

Single-lung transplantation: changes in the native not-transplanted lungs

We performed the quantitative CT scan comparison of the native not-transplanted lungs before (196 [85-282] days) and after the other lung was transplanted (data available for 12 of the 14 patients) (see the Additional File, Table E12). The increase in the inhomogeneity of the native not-transplanted lungs before and after surgery was strongly related to [^{18}F]FDG uptake (R^2=0.92, p<0.0001) (Figure 3). The inhomogeneities were mainly located at the interfaces of structures with different elasticity (Figure 4), mostly between the visceral pleura and the subpleural pulmonary units or near lesions caused by the primary lung disease.

During the surgical procedure the native not-transplanted lungs underwent one-lung ventilation for 197 [189-200] minutes with tidal volume of 8.2 [6.9-8.8] mL/kg, Positive End Expiratory Pressure (PEEP) ranging from 4 [2-5], plateau pressure up to 33 [27-35] cmH$_2$O, and respiratory rate 15 [13-18] breaths/min. Further details are reported in the Additional File, Table E4.

Discussion

The primary findings of this study are: 1) the grafts that developed PGD had similar [^{18}F]FDG uptake rates, systemic and BAL inflammatory markers compared to patients who did not; 2) both the grafts and the native not-transplanted lungs presented a [^{18}F]FDG uptake rate significantly greater than healthy subjects but lower than ARDS patients 3) PGD patients in the first hours after transplantation presented higher heart rate, central venous pressure, fluid balance and shunt fraction than patients who did not, associated with a CT scan morphology suggesting the presence of increased lung edema (larger amount of not-inflated tissue and less well-inflated tissue). As additional finding, we documented that native not-transplanted lungs deteriorated in the post-operative period, compared to the pre-operative evaluation.

This study was designed to test the hypothesis that the edema of PGD patients is by inflammatory origin and is associated with an increased metabolic activity, measured by [^{18}F]FDG uptake rate. If our hypothesis holds true, the pathogenesis of increased alveolo-capillary permeability, lung collapse and hypoxemia would be similar in ARDS and PGD, the two diseases differing only for the triggering events. PGD is defined only according to clinical criteria (lung infiltrates associated with hypoxemia) without taking in account the pathogenesis of the syndrome, which is always supposed to be immune-mediated. There is extensive evidence of activation of the inflammatory cascade in PGD patients but the present data suggest that hemodynamic factors may play a role, at least in a subgroup of patients and, maybe, a new definition taking in account the pathogenesis is warranted. Even if the lungs in patients who developed PGD showed an increased

[^{18}F]FDG uptake rate compared to healthy lungs, we did not detect differences with patients who did not develop PGD. Moreover, the activation of inflammation, at least neutrophilic, was lower if compared to ARDS, known to be characterized by widespread lung inflammation. We based our hypothesis on results of experimental transplants, in which the PGD was associated with oxidative stress, increased inflammatory cytokines and ischemia-reperfusion injury[19–21]. However, in our patients the [^{18}F]FDG uptake rate in grafts that developed PGD was the same as in those that did not despite morphological differences in the quantitative analysis of CT scan (increased not inflated tissue and decreased well inflated tissue). We saw no significant increase in Reactive C protein or in leukocytes count or differences in broncho-alveolar fluid lavage in patients who did or did not develop PGD and we were unable to find significant differences in BAL inflammatory mediators between patients who developed and did not develop PGD, except for a trend toward an increase in TIMP1 in PGD patients. The [^{18}F]FDG uptake rate is just a nonspecific marker of inflammation, described in literature reflecting mostly, but not exclusively, the metabolic activity of neutrophils[19]. We may speculate that simply different inflammatory pathways non related to [^{18}F]FDG uptake rate are involved in PGD or may raise the hypothesis that factors beyond inflammation are involved in PGD. In fact, Jones HA et al[11] found that rejection alone did not increase [^{18}F]FDG PET signal in cohort of patients studied after a median of 2 months post-transplant suggesting that [^{18}F]FDG PET is not sensitive to the cellular mechanisms which are at the basis of rejection. In our patient population a series of hemodynamic signs in patients who developed PGD suggested that, to different extents, the grafts in our patients who developed PGD actually had more edema than those who did not develop the dysfunction. Unfortunately, although we provided invasive hemodynamic monitoring, we planned no systematic follow-up with echocardiography. Therefore we cannot distinguish whether the edema was due to slight impairment of cardiovascular function and/or to inappropriate fluid loading in an inflamed lung. The contribution of hemodynamic factors to PGD had been reported in literature. In particular a retrospective study on a court of 161 transplant patients reported that a worse left ventricle diastolic function during evaluation for lung

transplantation was associated with a significantly increased risk of grade 3 PGD[22]. Of note, the two hypotheses on PGD pathogenesis are not mutually exclusive as the increase of capillary permeability due to inflammation may generate edema in presence of an only slightly increased hydrostatic component.

Although the native not-transplanted lungs had the same [^{18}F]FDG uptake rates as the grafts, the causes are not necessarily the same. In fact, native not-transplanted lungs showed a worse inflation status and a less homogeneous parenchyma than grafts. Furthermore, the CT scan characteristics deteriorated in the post-operative period, compared to the pre-operative evaluation. The native not-transplanted lungs, during single-lung transplantation, underwent substantial stress during the one-lung ventilation (median plateau pressure greater than 30 cmH$_2$O, lasting 197 [189-200] minutes). It is tempting to speculate that in these lungs the higher [^{18}F]FDG uptake rate is an expression of ventilator-induced lung injury (VILI)[23,24]. Interestingly, [^{18}F]FDG uptake rate in these lungs increased mainly at the interfaces of lung structures with different elasticity, i.e. at the visceral pleura where stress and strain are multiplied or near lesions already present before surgery. The spatial distribution of lesions in the early phase of experimental VILI in animals was similar[25].

Our study presents some main limitations. The first one is the small sample size of population. As previously showed, our population was enough only to state that the lungs of patients who developed PGD were not so inflamed as lungs of ARDS patients. We also could not include in the study patients who needed non-invasive ventilation at the time of CT-PET study, because we have not this support in the CT-PET room but only patients completely dependent or independent from mechanical ventilation.

All patients we studied had a favorable outcome in term of survival: none died in the first 28 days after transplantation, all patients were discharged from hospital. Probably our sample was too small to infer something about mortality and outcome in general in patients after lung transplantation.

CT-PET study was performed after a median time of 5 days (not different between patients with and without PGD) while PGD developed (by definition) within 3 days. We believe that, if any difference existed in [18F]FDG uptake rate between the two groups, it would have been carried on for the two days between PGD diagnosis and PET scanning, as the data obtained from the CT scan analysis.

Conclusions

The grafts of patients who developed PGD had similar [18F]FDG uptake rates, systemic and BAL inflammatory markers compared to grafts of patients who did not, suggesting that non-inflammatory mechanisms (i.e. the hydrostatic component combined with a slight inflammation) may play a role in PGD development. Early after lung transplantation, [18F]FDG uptake rate was significantly greater than in healthy subjects but lower than in ARDS patients.

What we already know about this topic

•	Primary graft dysfunction (PGD) is the leading cause of early mortality after lung transplantation.

•	Inflammation could play a central role in PGD development.

What this article tells us that is new

•	There were no differences in [18F]FDG uptake rates, systemic and BAL inflammatory markers between patients who developed PGD and patients who did not.

•	After lung transplantation, both the grafts and the native not-transplanted lungs, in case of single lung transplantation, presented an [18F]FDG uptake rate greater than healthy subjects, but lower than ARDS patients.

•	In single lung transplantation, the native not-transplanted lung worsened in post-operative period, after one lung ventilation, if compared to the pre-operative CT scan.

Notes

References

1. Yusen RD, Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: " Thirtieth Adult Lung and Heart-Lung Transplant Report--2013; focus theme: age." J Heart Lung Transplant Off Publ Int Soc Heart Transplant. 2013 Oct;32(10):965–78.

2. Shah RJ, Diamond JM, Cantu E, Lee JC, Lederer DJ, Lama VN, et al. Latent class analysis identifies distinct phenotypes of primary graft dysfunction after lung transplantation. Chest. 2013 Aug;144(2):616–22.

3. Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant Off Publ Int Soc Heart Transplant. 2005 Oct;24(10):1454–9.

4. Christie JD, Bellamy S, Ware LB, Lederer D, Hadjiliadis D, Lee J, et al. Construct validity of the definition of primary graft dysfunction after lung transplantation. J Heart Lung Transplant Off Publ Int Soc Heart Transplant. 2010 Nov;29(11):1231–9.

5. Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. Am J Respir Crit Care Med. 2007 Mar 1;175(5):507–13.

6. Diamond JM, Meyer NJ, Feng R, Rushefski M, Lederer DJ, Kawut SM, et al. Variation in PTX3 is associated with primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med. 2012 Sep 15;186(6):546–52.

7. Sayah DM, Mallavia B, Liu F, Ortiz-Munoz G, Caudrillier A, DerHovanessian A, et al. Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med. 2015 Feb 15;191(4):455–63.

8. Suzuki Y, Cantu E, Christie JD. Primary graft dysfunction. Semin Respir Crit Care Med. 2013 Jun;34(3):305–19.

9. Yang Z, Sharma AK, Linden J, Kron IL, Laubach VE. CD4+ T lymphocytes mediate acute pulmonary ischemia-reperfusion injury. "PG - 695-702; discussion 702." J Thorac Cardiovasc Surg. 2009 Mar;137(3).

10. Bellani G, Guerra L, Musch G, Zanella A, Patroniti N, Mauri T, et al. Lung regional metabolic activity and gas volume changes induced by tidal ventilation in patients with acute lung injury. Am J Respir Crit Care Med. 2011 May 1;183(9):1193–9.

11. Jones HA, Donovan T, Goddard MJ, McNeil K, Atkinson C, Clark JC, et al. Use of 18FDG-pet to discriminate between infection and rejection in lung transplant recipients. Transplantation. 2004 May 15;77(9):1462–4.

12. Cressoni M, Chiumello D, Chiurazzi C, Brioni M, Algieri I, Gotti M, et al. Lung inhomogeneities, inflation and [18F]2-fluoro-2-deoxy-D-glucose uptake rate in acute respiratory distress syndrome. Eur Respir J. 2016 Jan;47(1):233–42.

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13. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 1983 Mar;3(1):1–7.

14. Gattinoni L, Pesenti A, Avalli L, Rossi F, Bombino M. Pressure-volume curve of total respiratory system in acute respiratory failure. Computed tomographic scan study. Am Rev Respir Dis. 1987 Sep;136(3):730–6.

15. Cressoni M, Gallazzi E, Chiurazzi C, Marino A, Brioni M, Menga F, et al. Limits of normality of quantitative thoracic CT analysis. Crit Care Lond Engl. 2013;17(3):R93.

16. Cressoni M, Cadringher P, Chiurazzi C, Amini M, Gallazzi E, Marino A, et al. Lung inhomogeneity in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med. 2014 Jan 15;189(2):149–58.

17. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012 Jun 20;307(23):2526–33.

18. R Core Team. R: A language and enviroment for statistical computing [Internet]. Vienna, Austria: R Fundation for Statistical Computing; 2016. Available from: www.R-project.org

19. Laubach VE, Kron IL. Pulmonary inflammation after lung transplantation. Surgery. 2009 Jul;146(1):1–4.

20. Sharma AK, Fernandez LG, Awad AS, Kron IL, Laubach VE. Proinflammatory response of alveolar epithelial cells is enhanced by alveolar macrophage-produced TNF-alpha during pulmonary ischemia-reperfusion injury. Am J Physiol Lung Cell Mol Physiol. 2007 Jul;293(1):L105-113.

21. Fiser SM, Tribble CG, Long SM, Kaza AK, Cope JT, Laubach VE, et al. Lung transplant reperfusion injury involves pulmonary macrophages and circulating leukocytes in a biphasic response. J Thorac Cardiovasc Surg. 2001 Jun;121(6):1069–75.

22. Porteous MK, Ky B, Kirkpatrick JN, Shinohara R, Diamond JM, Shah RJ, et al. Diastolic Dysfunction Increases the Risk of Primary Graft Dysfunction after Lung Transplant. Am J Respir Crit Care Med. 2016 Jun 15;193(12):1392–400.

23. Lohser J, Slinger P. Lung Injury After One-Lung Ventilation: A Review of the Pathophysiologic Mechanisms Affecting the Ventilated and the Collapsed Lung. Anesth Analg. 2015 Aug;121(2):302–18.

24. Kozian A, Schilling T, Schutze H, Senturk M, Hachenberg T, Hedenstierna G. Ventilatory protective strategies during thoracic surgery: effects of alveolar recruitment maneuver and low-tidal volume ventilation on lung density distribution. Anesthesiology. 2011 May;114(5):1025–35.

25. Cressoni M, Chiurazzi C, Gotti M, Amini M, Brioni M, Algieri I, et al. Lung inhomogeneities and time course of ventilator-induced mechanical injuries. Anesthesiology. 2015 Sep;123(3):618–27.

Figure captions

Figure 1: Lung distribution of [18F]FDG uptake rates along the sternum-vertebral axis

The figure shows the average [18F]FDG uptake rates ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min) along the sternum-vertebral axis. Lung CT slices were divided into 10 levels (as depicted) for the analysis. [18F]FDG uptake increases from level 7 to level 10.

Figure 2: The [18F]FDG uptake rates in patients with and without a clinical diagnosis of PGD.

The [18F]FDG uptake rate in two patients with no clinical diagnosis of PGD (right column) and two with PGD (left column). [18F]FDG uptake rates are similar in both groups. Lung areas with increased [18F]FDG uptake are mostly around chest tubes, vascular anastomoses and in subpleural regions.

Figure 3: [18F]FDG uptake rates and the increase in lung inhomogeneity before/after lung transplantation in native not-transplanted lungs.

The figure shows the average [18F]FDG uptake rates ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min) of the native not-transplanted lungs in relation to the changes in inhomogeneity (%), before and after single-lung transplantation. Greater changes in inhomogeneity in the not-transplanted lungs correlate with higher [18F]FDG uptake after transplantation of the contralateral lung.

[18F]FDG uptake rate ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min) $= 14.1 + 1.1 *$ change in inhomogeneity before-after transplantation (% of lung volume), R^2=0.92, p<0.0001.

Figure 4: Lung CT scan and false-colors map of inhomogeneity in native not-transplanted lungs before and after single-lung transplantation.

Examples of lung CT scan and false-colors map of inhomogeneity in the native not-transplanted lungs in four representative patients before and after single lung transplantation. Areas of inhomogeneity below the threshold of normal subjects (1.61) are colored in blue and areas of

inhomogeneity above the threshold are orange. The increase seems to originate from pre-operative

foci of inhomogeneity.

[18F]FDG uptake rate ($*10^{-4}mL_{blood}/mL_{tissue}/min$)

preoperative   postoperative
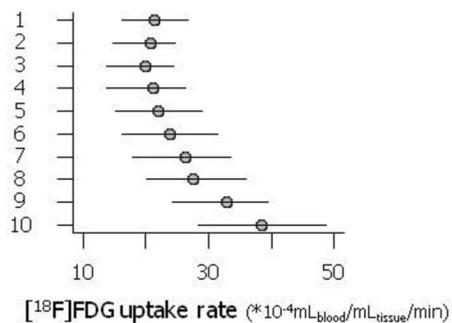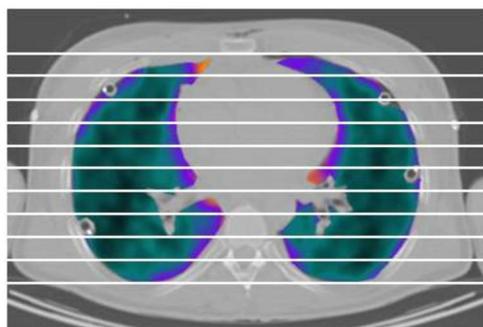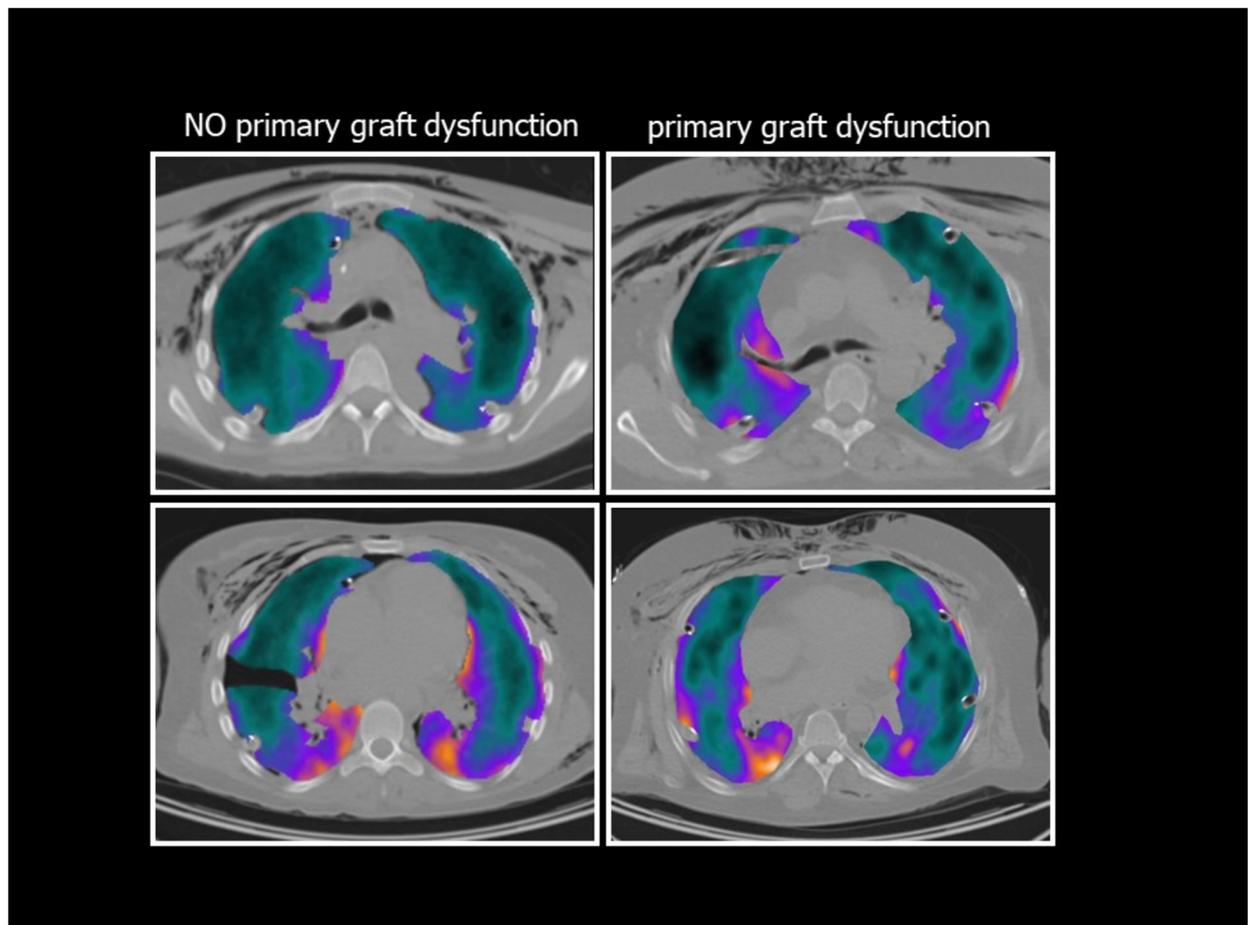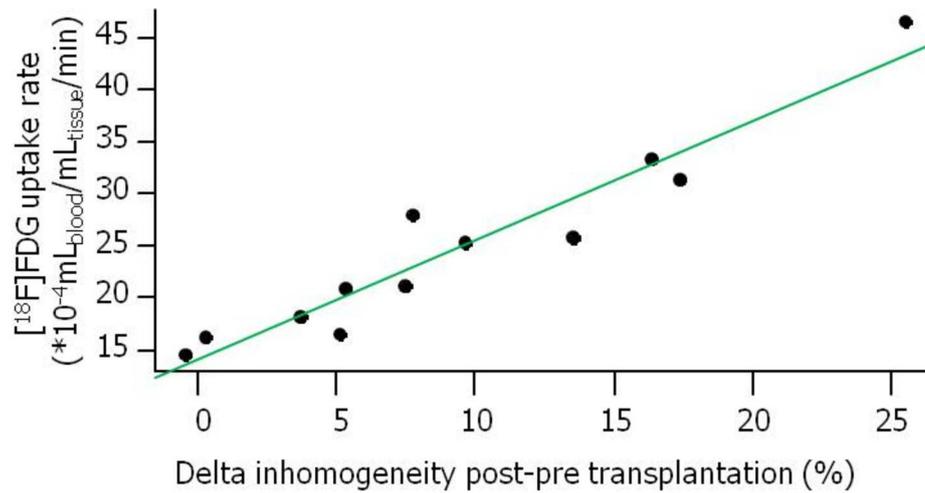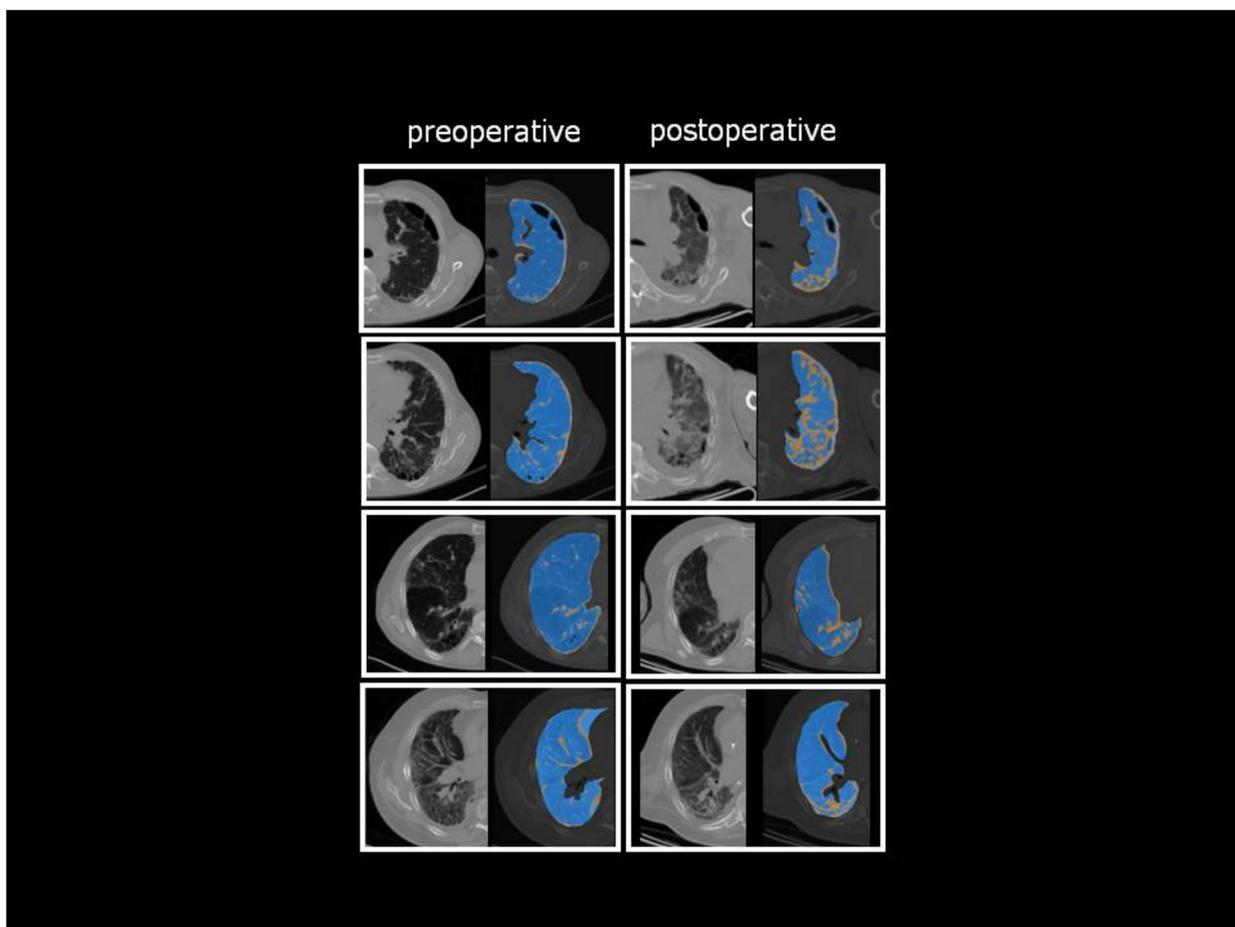
Inflammation and primary graft dysfunction after lung transplantation: CT-PET findings

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Additional File

Supplementary Methods

Informed consent

Informed consent was obtained before lung transplantation from each patient. A written permission of using the data collected was obtained.

Study protocol

Patients were enrolled before lung transplantation. Exclusion criteria were an age below 18 years and the impossibility to safely transport patients from the ICU to the Nuclear Medicine Unit. The admission to the lung transplantation waiting list, the pre-operative, intra-operative and post-operative management were conducted according to our institution's protocol.

Anaesthesia during lung transplantation

During surgery, anesthesia plan was set according to our institution protocol. Briefly, induction of general anaesthesia was performed with infusion of a Propofol bolus, and anaesthesia maintenance was achieved with Sevoflurane and continuous infusion of fentanyl; when the use of inhalatory anaesthesia was not possible (eg. during central venous-arterial extracorporeal support), TIVA with Propofol and Fentanyl continuous infusion was performed. During surgery, paralysis was maintained with either Rocuronium refracted bolus infusion or Cisatracurium continuous infusion.

Immunosuppressive therapy

The three-drug maintenance immunosuppressive regimen was set according to our institution protocol. Briefly, immunosuppressive therapy started immediately after transplantation. It includes a calcineurin inhibitor (tacrolimus), a purine synthesis antagonist (azathioprine) and a

glucocorticoid (methylprednisolone or prednisolone). The dose of tacrolimus was adjusted to the target of 0.075 mg/kg/day; during the following days, tacrolimus was adjusted according to blood level monitoring and renal function.

Pre-operative variables

Pre-operative parameters were assessed during patient's evaluation for admission to lung transplantation waiting list according to our institution's protocol. Data were collected retrospectively. Anthropometric measures (actual weight (kg), height (m), BMI (kg/m^2)), blood tests, pulmonary function tests including pulmonary scintigraphy, cardiovascular tests including cardiac ultrasonography and cardiac catheterization were recorded. In 12 out of 14 patients who underwent single lung transplantation, pre-operative CT study was collected to allow imaging analysis of native not-transplanted lung, performed as explained in "*CT scan analysis*". Of note, right heart function was globally conserved as shown by right heart catheterization (see Table E10, "preoperative evaluation" column) and cardiac ultrasonography. Cardiac ultrasonography documented a normal kinesis of both the ventricles, in absence of valvular defects. Moreover, angiographic examination of heart was performed, in order to exclude from waiting list patients affected by ischemic disease.

Intra-operative variables

Intra-operative data were collected by on dedicated data sheets. Three standard timepoints of data collection were established: just before surgical incision, at clamping of the first artery and at the end of surgical procedure. Invasive hemodynamic monitoring was provided by placement of a pulmonary-artery catheter at induction through which right heart, pulmonary and wedge pressures were recorded. Other hemodynamic parameters considered were: arterial pressure, fluid balance, infusion of inotropes and/or vasopressors and need of extracorporeal support. Ventilator parameters

(tidal volume, respiratory rate, minute ventilation, inspiratory fraction of oxygen, positive end-expiratory pressure, mean airway pressure, plateau pressure, peak pressure) and arterial and mixed venous blood gas analysis (pH, pO_2, oxygen saturation, pCO_2, PaO_2/FiO_2, base excess, bicarbonate, lactate) were collected.

Post-operative variables

In the intensive care unit, data from arterial and mixed venous blood gas analysis (pH, pO_2, oxygen saturation, pCO_2, PaO_2/FiO_2, base excess, bicarbonate, lactate), temperature, hemodynamic (arterial pressure, central venous pressure, pulmonary pressure, wedge pressure, cardiac output) and ventilator parameters (tidal volume, respiratory rate, minute ventilation, inspiratory fraction of oxygen, positive end-expiratory pressure, mean airway pressure, plateau pressure, peak pressure) were collected at the admission, after the first stabilization of the patient (approximately after the first 6 hours), then once a day every day until CT-PET study. From the first postoperative day, a chest X-ray was performed daily, in order to assess PGD development. Laboratory exams, including blood cell count (leukocytes, erythrocytes and platelet count), hemoglobin, hematocrit, differential white blood cell count, serum glucose, lactate dehydrogenase, amylase, serum protein concentration, standard liver panel (albumin, aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase, total bilirubine), kidney function tests (creatinine, blood urea nitrogen), electrolytes (sodium, potassium, chloride, total calcium), coagulation tests (activated partial thromboplastin time, International normalized ratio), C-reactive protein were collected daily from the ICU admission to the CT-PET study.

Outcome variables

Length of ICU stay (days), length of hospital stay (days) and days of mechanical ventilation were considered as general indicators of outcome. Gas exchange parameters ($PaCO_2$ (mmHg) and

PaO$_2$/FiO$_2$), pulmonary function tests (FEV1 (%) and FEV1/FVC (%)) and assessment of cardiovascular function (distance walked at the six minutes walk test (meters)) at discharge were recorded as functional indicators of outcome.

Broncho-alveolar lavage (BAL)

Bronchoscopy was completed at least 4 hours ahead the imaging study to avoid interference of the procedure on the imaging study. One of the staff physician member of the study team executed the bronchoscopy. The procedure was performed using a fiber-optic bronchoscope with separate channels for saline instillation and sample withdrawal[1]. The bronchoscope was introduced through the endotracheal/tracheostomy tube in mechanically ventilated patients and through the mouth in spontaneous breathing patients [2].

Fraction of inspired oxygen was transiently increased to 100% for the duration of the procedure.

A single bronchoaspirate sample was obtained for microbiological analysis. The thoracic surgeon visually inspected the bronchial anastomosis. The bronchoscope was wedged into a distal bronchial branch within the right middle lobe and bronchoalveolar lavage (BAL) was performed. Five separate 30 mL aliquots of 0.9% NaCl at 21°C were gently hand instilled and withdrew after few seconds. The BAL recovery averaged 120±20 mL. The BAL aliquots were transported immediately to the laboratory for processing. The fluid was pooled and filtered through gauze moistened with 0.9% NaCl to remove mucus. The total cells count was performed with a hemacytometer. Differential cells count was performed on cytospin preparations stained with modified Wright-Giemsa. The total protein content of the BAL fluid was measured on an aliquot of the supernatant using the bicinchoninic acid method. A contemporary blood sample was obtained to measure total plasma protein dosage.

Evaluation of mediator concentration in BAL.

BAL samples were centrifugated at 1500 rpm for 15 min at 4°C (Haereus Multifuge X3R, Thermo Fisher Scientific Inc., Cambridge, UK). Supernatants were collected and stored at - 20°C for mediator concentration assessment.

A panel of soluble proteins relevant to inflammatory response and epithelial/glycocalyx damage was evaluated using custom-designed immunoassays based on xMAP® Technology (R&D Systems, Inc., Minneapolis, MN, USA) or commercial available enzyme-linked immunosorbent assays (ELISA) (R&D Systems) (see Table below for sensitivity of all tests used). Fluorescence detection was performed using Luminex 200 (Luminex corporation, Austin, CA), while absorbance was measured by ultraviolet–visible spectroscopy (Titertek Multiscan).

Sensitivity and low detection limits of the tests used to evaluate mediator concentration in BAL samples.

Analyte symbol	Analyte name	Assay	Sensivity, pg/ml	Assay range, pg/ml
bFGF	basic fibroblast growth factor	xMAP Technology	6.5	1.89 - 460
CCL2/MCP-1	C-C motif chemokine 2 / monocyte chemotactic protein 1	xMAP Technology	9.9	31.9 - 7,740
CXCL8/IL-8	C-X-C motif chemokine ligand 8 / interleukin-8	xMAP Technology	1.8	4.4 - 1,060
CXCL10/IP-10	C-X-C motif chemokine ligand 10 / IFN-induced protein-10	xMAP Technology	1.18	2.8 - 690
IL-1 beta	interleukin 1 beta	xMAP Technology	0.8	16.3 - 3,960
IL-6	interleukin 6	xMAP Technology	1.7	4.5 - 1,100
MMP7	matrix metallopeptidase 7	ELISA	0.094 *10^3	0.2 - 10 *10^3
RAGE	advanced glycosylation end-product specific receptor	ELISA	16.14	78.0 - 5,000
SERPINE1/PAI-1	serpin family E member 1 / plasminogen activator inhibitor-1	ELISA	0.142 *10^3	0.3 - 20 *10^3
TIMP1	tissue metallopeptidase inhibitor 1	xMAP Technology	3.42	54.7 - 13,280
TNF-alpha	tumor necrosis factor alpha	xMAP Technology	1.2	8.9 - 2,160

CT-PET image acquisition

CT-PET study was performed according to a previous published protocol[3].

During CT-PET study (Biograph 64 TruePoint, Siemens, Germany®) patients underwent a dynamic CT acquisition with low-dose scan protocol (performed with automatic control of tube current 60-100 mAs at 120 kV, beam collimation 24x1.2). Thereafter a dynamic PET acquisition started with a bolus of [18F]fluoro-2-deoxy-D-glucose ([18F]FDG) injected in about 30 s (300 MBq, range 260–390 MBq). Dynamic PET frames (24x5s, 6x180s, 7x300s) were acquired over 60 minutes. The length of time of acquisition made irrelevant a possible effect of hypoperfusion. The total absence of perfusion can not be, anyway, overcome. During PET imaging blood samples were sequentially drawn at 0.3, 0.5, 0.7, 1.0, 1.3, 1.7, 2, 4, 8, 12, 27, 42 and 57 minutes from a central vein to measure [18F]FDG plasma content. Blood sample-activity concentrations (Bq/mL) were assessed with a well counter (WIZARD, Perkin-Elmer) cross-calibrated with the PET scanner.

PET image analysis

PET images were reconstructed on 74 slices (matrix 128x128; voxel size = 3x3x3 mm). The graphical Patlak approach was applied to dynamic PET volumes to estimate voxel by voxel [18F]FDG uptake rate: after skipping PET frames performed during the first 8 minutes of acquisition, for each time point (from 10 to 60 minutes), voxel-by-voxel, lung activity normalized to blood activity versus integral of plasma activity normalized to blood activity was plotted and the slope of the linear part of the graph represents [18F]FDG voxel uptake rate ($mL_{blood}/mL_{tissue}/min$). This parametric volume was thereafter registered to the resolution of CT scan (512x512) in order to have no loss of information when comparing PET data with CT data.

Patlak analysis

Patlak model[4] assumes a blood-plasma compartment, a reversible tissue region with an arbitrary number of compartments and one or more irreversible tissue regions. The model assumes linear

transfer kinetics. It can be mathematically demonstrated that the slope of the plot of the ratio (solute concentration in the tissue/blood concentration) at the timed of interest (y axis) versus the ratio (integral of plasma concentration respect to time/plasma concentration) at the respective times (x axis) represents the influx constant of tracer into the irreversible compartment ([^{18}F]FDG uptake rate, $mL_{blood}/mL_{tissue}/min$). The Patlak model was developed to describe the transfer across the blood-brain barrier and can be applied to nuclear medicine imaging as the traces [^{18}F]FDG is irreversibly trapped into cells.

CT scan analysis

CT scans performed the day of CT-PET study and pre-operative CT scans of patients who underwent single lung transplantation were analyzed as follows.

Lung profiles were manually delineated on each lung CT scan image excluding the hilar structures and eventual pleural effusion. Thereafter quantitative analysis of CT scan images was performed with a dedicated software (Soft-E-Film, www.softefilm.eu). As CT scan measures density, assuming that the lung is composed by two compartments with very different densities – lung tissue with a density close to the one of water (0 HU) and gas with a density of -1000 HU – it is possible to compute tissue and gas content in each voxel:

Voxel gas content (mL) = (CT number (HU)/-1000)*voxel volume (mL)

 Voxel tissue content (g) = voxel volume (mL) – voxel gas content (mL) [as water density is 1 g/mL]

CT scan cannot differentiate between lung tissue, blood and edema, which all have densities close to that of water. Lung tissue was classified according to its gas/tissue content as not (CT number >-100), poorly inflated (-100>CT number>-500), well inflated (-500>CT number>-900) and over inflated (CT number<-900).

Sternum-vertebral division

We divided each lung in 10 *sections* equally spaced along the horizontal axis (apex to base) and dividing each *section* in 10 equally spaced *levels* along the vertical axis (sternum to vertebra). Levels with the same number were merged.

Computation of lung inhomogeneities

Lung inhomogeneities were determined as previously described[5]. We applied the Mead's model[6] to whole lung CT scan images of lung parenchyma. A homogenous lung, when kept open by a force (transpulmonary pressure) would have the same gas/tissue in all its regions. If a lung region expands less/does not expand, the surrounding lung regions would expand more to vicariate the non-expanding one and, locally, stress and strain would be increased. Regional lung expansion can be measured as regional gas/tissue ratio in two contiguous lung regions: if two neighbor lung regions present different inflation status, one is acting as a "stress raiser" on the other, as the lung is kept inflated by the transpulmonary pressure. We defined "extent" of lung inhomogeneities the fraction of lung volume whose inhomogeneities were greater than the 95th percentile of the inhomogeneities computed in normal subjects (1.61). We defined "intensity" the average value of lung inhomogeneities included in that fraction.

Since CT scan images are composed by voxels whose dimensions depend both on the CT scan hardware and on the setting for image reconstruction, we decided to produce a lung inhomogeneities map with dimensions 1:1 to the original CT scan map but using as a "basic dimension" the acinar volume (186 mm^3). The following algorithm was applied to compute lung inhomogeneities:

1) The original CT scan was filtered with a gaussian filter with a radius equal to the radius of human acinus (volume 186 mm^3, radius 2.41 mm)[7]. While average is a square filter and

takes into the same account near and far voxels, Gaussian filters exponentially decreases weight of far voxels.

2) We defined a spherical crust centered on the interested voxel starting from 2.41 mm (acinus radium) and ending at 3.675 mm (acinus radius*0.5). The spherical crust included approximately ~80 voxels.

3) We computed a vector of lung inhomogeneities dividing the filtered gas fraction in each of the voxels included, at least partially, in the spherical crust and the filters value of the central voxel.

4) We wrote the maximum of the vector in the lung inhomogeneities map.

Hemodynamic and gas exchange variables computation

The following equation was used for the computation of right-to-left shunt fraction (fraction of cardiac output):

Shunt fraction = $(CcO_2 - CaO_2)/(CcO_2 - CvO_2)$

where CcO_2 is the capillary oxygen content (mL/dL), CaO_2 is the arterial oxygen content (mg/dL), and CvO_2 is the venous oxygen content (mL/dL). The capillary oxygen content (CcO_2) was computed as

$CcO_2 = [PAO_2 \text{ (mmHg)} * 0.003] + Hb \text{ (g/dL)} * 1 * 1.39$

where PAO_2 is the alveolar partial pressure of oxygen and was estimated as $PAO_2 = FiO_2 * (760 - 47) - PaCO_2/(\text{Respiratory Quotient})$ using a Respiratory Quotient of 0.8 and Hb is the blood concentration of hemoglobin.

The arterial oxygen content (CaO_2) was computed as :

$CaO_2 = [PaO_2 \text{ (mmHg)} * 0.003] + Hb \text{ (g/dL)} * SaO_2 * 1.39$

where PaO_2 is the arterial partial pressure of oxygen, Hb is the blood concentration of hemoglobin, and SaO_2 is the arterial oxygen saturation.

The venous oxygen content (CvO_2) was computed as

$$CvO_2 = [PvO_2 \text{ (mmHg)} * 0.003] + Hb \text{ (g/dL)} * SvO_2 * 1.39$$

where PvO_2 is venous partial pressure of oxygen, Hb is the blood concentration of hemoglobin, and SvO_2 is the venous oxygenation saturation.

Consequently, artero-venous difference in oxygen content was computed as the difference between the arterial oxygen content (CaO_2) and the venous oxygen content (CvO_2):

$$\text{a-vO}_2 \text{ diff} = CaO_2 - CvO_2$$

Patient classification

Patients were classified in those who developed primary graft dysfunction (PGD) and those who did not develop PGD. The clinical diagnostic criteria for PGD were defined by the International Society of Heart and Lung Transplantation in 2005[8]. The PGD is a syndrome of acute lung injury that occurs within the first 72 hours after lung transplantation. It is characterized by the presence of infiltrates consistent with diffuse pulmonary edema at the chest X-ray and progressive hypoxemia (PaO_2/FiO_2 <300). The severity of PGD is graded based on the PaO_2/FiO_2 ratio:

- Grade 1 – $PaO_2/FiO_2 > 300$
- Grade 2 – PaO_2/FiO_2 between 200 and 300
- Grade 3 – $PaO_2/FiO_2 < 200$

Exclusion criteria are: hyperacute rejection, venous anastomotic obstruction, cardiogenic pulmonary edema and pneumonia (both viral and bacterial).

Supplementary Results

Demographic and anthropometric characteristics of patients

In Table E1 we summarize the main demographic and anthropometric characteristics of the patients. The mean waiting list time was 214[93-322] days. Ten double-lung transplantations were performed. Intra-operative extracorporeal support was necessary during two double-lung transplantations and one single-lung transplantation.

Table E1

Patients	Sex	Age	BMI	Indication for lung transplantation	LTx
1	M	64	23	Idiopathic pulmonary fibrosis	RLTx
2	M	64	25	Interstitial lung disease in Hermansky Pudlak syndrome	RLTx
3	M	65	22	Chronic obstructive pulmonary disease + usual interstitial pneumonia	LLTx
4	M	41	28	Cystic fibrosis	DLTx
5	F	23	24	Cystic fibrosis	DLTx
6	M	50	25	Chronic obstructive pulmonary disease/emphysema	DLTx
7	M	47	31	Cystic fibrosis	DLTx
8	M	67	28	Idiopathic pulmonary fibrosis	RLTx
9	M	55	21	Idiopathic pulmonary fibrosis	LLTx
10	M	58	35	Idiopathic pulmonary fibrosis	LLTx
11	M	66	22	Idiopathic pulmonary fibrosis	LLTx
12	M	60	31	Idiopathic pulmonary fibrosis	RLTx
13	M	65	26	Idiopathic pulmonary fibrosis	LLTx
14	F	30	28	Histiocytosis X	DLTx
15	F	58	33	Idiopathic pulmonary fibrosis	RLTx
16	F	56	27	Idiopathic pulmonary fibrosis	RLTx
17	F	60	25	Non-specific interstitial pneumonia	RLTx
18	M	62	29	Idiopathic pulmonary fibrosis	RLTx
19	M	45	25	Cystic fibrosis	DLTx
20	M	42	26	Cystic fibrosis	DLTx
21	M	32	32	Histiocytosis X	DLTx
22	M	36	26	Cystic fibrosis	DLTx
23	M	64	29	Idiopathic pulmonary fibrosis	LLTx
24	M	47	23	Systemic sclerosis	DLTx

Table E1: Demographic and anthropometric characteristics of the patients. M: male; F: female; BMI: body mass index; LTx: lung transplantation; RLTx: right lung transplantation; LLTx: left lung transplantation; DLTx: double lung transplantation.

Demographic and anthropometric characteristics of donors

In Table E2 we summarize the main demographic and anthropometric characteristics of the donors together. Only two donors had a history of smoke. They also had an abnormal chest X-ray and tracheobronchial secretions. Donors without an abnormal chest X-ray and/nor tracheobronchial secretions were seven. The mean time of mechanical ventilation was 2[1-3] days. Hypotension occurred in 13 donors and 21 received vasopressors. The best PaO_2/FiO_2 was 499[442-549], while the last PaO_2/FiO_2 before lung was 454[371-512].

Table E2

Patients	Sex	Age	BMI	Cause of death
1	M	49	23	Cerebrovascular accident
2	M	42	25	Cerebrovascular accident
3	M	19	22	Trauma
4	M	33	28	Cerebrovascular accident
5	F	56	24	Cerebrovascular accident
6	F	70	25	Cerebrovascular accident
7	M	53	31	Cerebrovascular accident
8	M	61	28	Cerebrovascular accident
9	M	56	21	Trauma
10	M	17	35	Cerebrovascular accident
11	F	35	22	Trauma
12	M	65	31	Cerebrovascular accident
13	F	39	26	Cerebrovascular accident
14	M	54	28	Cerebrovascular accident
15	M	60	33	Cerebrovascular accident
16	M	32	27	Trauma
17	M	25	25	Cerebrovascular accident
18	F	53	29	Cerebrovascular accident
19	M	38	25	Cerebrovascular accident
20	M	33	26	Cerebrovascular accident
21	M	52	32	Cerebrovascular accident
22	M	45	26	Cerebrovascular accident
23	M	66	29	Trauma
24	M	60	23	Cerebrovascular accident

Table E2: Demographic and anthropometric characteristics of donors. M: male; F: female; BMI: body mass index.

Cold and warm ischemia

In Table E3 we show cold and warm ischemia. Cold ischemia is defined as the time of hypothermic preservation beginning when the organ is cooled with a cold perfusion solution after organ procurement surgery. It ends after the tissue reaches physiological temperature during implantation procedures. Warm ischemia time starts with completion of surgical anastomosis. Cold ischemia time in Graft1, obviously, tend to be lower than in Graft 2 (p=0.08). Interestingly, warm ischemia time is longer in Graft in single lung transplantation than Graft 1 in double lung transplantation.

Table E3

	Double lung transplantation (10 patients)		Single-lung transplantation (14 patients)
	Graft 1	**Graft 2**	**Graft**
Cold ischemia (min)	325 [223-375]	444 [391-512]	269 [230-290]
Warm ischemia (min)	77 [75-78]	86 [74-100]	100* [91-106]

Table E3: cold and warm ischemia time. Data are presented as median[IQR]. *p<0.05. Mann-Withney test.

One lung ventilation during single lung transplantation

Table E4 summarizes the ventilation parameters during one lung ventilation of the native not-transplanted lungs during single-lung transplantation surgical procedure. The median period of one lung ventilation was 197[189;200]minutes. Tidal volumes were of 8.2[6.9-8.8] and 8.2[7.8-8.6]mL/kg, respectively. As a consequence, abnormally high pressures developed with further injury to an already diseased lung.

Table E4

	Baseline	Clamp	De-clamp
Tidal volume (mL/kg)	9.5 [8.6-10.3]	8.2 [6.9-8.8]	8.2 [7.8-8.6]
Respiratory rate (breaths/min)	14 [12-16]	15 [13-18]	14 [12-18]
Peak pressure (cmH$_2$O)	27 [25-34.3]	35 [30.8-36]	35 [33.5-37.5]
Plateau pressure (cmH$_2$O)	25 [23.9-27]	33 [26.9-34.5]	30 [27-34.5]
Mean airways pressure (cmH$_2$O)	9 [8.7-10]	12 [12-15.8]	14 [12-16.8]
PEEP (cmH$_2$O)	4 [3-5]	4 [2-5]	5 [2-6]
FiO$_2$	0.60 [0.60-0.64]	0.75 [0.59-0.93]	0.62 [0.59-0.89]
EtCO$_2$ (mmHg)	34 [32-38]	33 [30-41]	30 [25-36]

Table E4: One lung ventilation parameters during single-lung transplantation. Baseline is the time just before surgical incision, after induction of anaesthesia; both lungs were ventilated at this timepoint. At the following timepoint, one lung ventilation was performed. Clamp is the time at which the first artery clamp occurred; de-clamping means the time at which the graft is re-perfused. Data are presented as median[IQR]. PEEP: positive end-expiratory pressure; FiO$_2$: fraction of inspired oxygen; EtCO$_2$: end-tidal carbon dioxide.

Severity of primary graft dysfunction

In Table E5 we summarize the severity of PGD according to the criteria defined by the International Society of Heart and Lung Transplantation in 2005 [8]. PGD was diagnosed in three patients who underwent double lung transplantation. Four patients improved their clinical condition from 24 to 72 hours after surgery while other four had the same PGD grade diagnosis over time. Only patient 8 had a diagnosis of PGD grade 1 at 24 hours and then got worse. At 72 hours after surgery, most PGD patients have a grade 2 diagnosis.

Table E5

Patient	Lung transplatation	24 hours after LTx	48 hours after LTx	72 hours after LTx
1	RLTx	2	2	2
2	DLTx	3	3	3
3	LLTx	2	2	2
4	LLTx	3	2	2
5	RLTx	3	2	2
6	RLTx	3	2	2
7	RLTx	3	3	3
8	DLTx	1	3	2
9	DLTx	3	2	2

Table E5: severity of PGD. LTx: lung transplantation; RLTx: right lung transplantation; LLTx: left lung transplantation; DLTx: double lung transplantation.

Systemic inflammatory indexes

Table E6 summarizes systemic inflammatory parameters in patients who developed and did not develop PGD. Timepoints are considered from admission in the ICU (timepoint '0'), after stabilization (timepoint '6') and once a day every day for the first 72 hours. Considering Reactive C Protein (mg/dL), patients who developed PGD have higher values at 72 hours from transplantation (12.6[11.7-16.1] versus 7.8[6.6-8.9], p=0.002).

Table E6

	Timepoint	0	6	24	48	72
Leukocytes (*10³cells/mm³)	*PGD*	14.9 [12.1-22.1]	14.9 [10.2-17.0]	14.1 [12.4-21.5]	17.0 [14.6-18.1]	12.4 [10.5-23.8]
	NO PGD	13.4 [10.4-16.0]	12.7 [11.3-14.5]	15.0 [13.2-17.6]	15.9 [13.9-18.5]	12.8 [10.7-16.0]
Reactive C Protein (mg/dL)	*PGD*	NA	NA	16.4 [14.1-17.8]	16.5 [16.0-23.3]	12.6* [11.7-16.1]
	NO PGD	NA	NA	13.4 [11.6-15.3]	14.0 [11.5-19.8]	7.8 [6.6-8.9]
Platelets (*10³cells/mm³)	*PGD*	183 [149-261]	163 [111-237]	152 [127-214]	151 [110-191]	136 [121-162]
	NO PGD	126 [114-164]	130 [106-159]	137 [108-156]	145 [102-172]	126 [106-166]

Table E6: systemic inflammatory parameters between patients who developed vs patients who didn't develop PGD. Data are presented as median[IQR] and compared with Mann Whitney test. *=p<0.05 PGD versus NO PGD.

Broncho alveolar lavage inflammatory indexes

Table E7

	No primary graft dysfunction (15 patients)	Primary graft dysfunction (9 patients)	p-value
Granulocytes (%)	10 [5-19]	50 [30-90]	0.10
Lymphocytes (%)	5 [3-10]	5 [5-10]	0.70
Macrophages (%)	80 [40-90]	45 [15-65]	0.14
Proteins (mg/dL)	17.2 [12.4-24.5]	22.9 [14.5-73.2]	0.44
Proteins BAL/plasma ratio	2.7 [2.2-4.6]	4.8 [3.5-5.9]	0.27

Table E7: Broncho-alveolar lavage cells in patients who developed PGD and those who did not. Data are median[IQR]. Mann-Whitney test. BAL, broncho-alveolar lavage.

Table E7a

	No primary graft dysfunction (7 patients)	Primary graft dysfunction (6 patients)	p-value
Granulocytes (%)	30 [30;50]	8 [5;55]	0.46
Lymphocytes (%)	5 [5;5]	5 [1.5;5]	0.41
Macrophages (%)	60 [45;70]	90 [40;90]	0.41
Proteins (mg/dL)	15.8 [14.7;30.0]	17.2 [15.1;28.6]	0.94
Proteins BAL/plasma ratio	4.8 [4.2;5.5]	3.2 [2.4;5.6]	0.52

Table E7a: Broncho-alveolar lavage cells in patients who developed PGD and those who did not, ONLY in patients with negative BAL culture. Data are median[IQR]. Mann-Whitney test. BAL, broncho-alveolar lavage.

Table E8

	Primary graft dysfunction (N=12)	No primary graft dysfunction (N=22)	P value
bFGF (pg/mL)[9]	250 [222 - 297]	235 [220 - 261]	0.38
CXCL8/IL-8 (pg/mL)[10]	774 [261 - 1462]	1562 [416 - 4153]	0.44
CXCL10/IP-10 (pg/mL)[11]	38 [17 - 111]	24 [20 - 36]	0.37
CCL2/MCP-1 (pg/mL)[9]	997 [416 - 2030]	417 [310 - 717]	0.20
MMP7(ng/mL)[12]	14 [9 – 29]	8 [3 – 13]	0.13
RAGE (ng/mL)[9]	6 [2 – 26]	5 [3 – 9]	0.82
TIMP1 (ng/mL)[9]	37 [16 – 244]	14 [6 – 28]	0.06
IL-6 (pg/mL)[13]	66 [20 - 175]	33 [13 - 134]	0.82
IL-1β (pg/mL)[10]	12 [1 - 32]	7 [4 - 92]	0.34
TNF-α (pg/mL)[10]	4 [2 - 11]	3 [2 - 7]	0.61
SERPINE1/PAI-1 (pg/mL)[9]	2442 [243 - 9652]	1089 [219 - 1603]	0.53

Table E8: Mediators BAL concentration. Data are median [IQR]. Wilcoxon Test. bFGF: fibroblast growth factor 2; CXCL8/IL-8: C-X-C motif chemokine ligand 8/interleukin 8; CXCL10/IP-10: C-X-C motif chemokine ligand 10/Interferon gamma-induced protein 10; CCL2/MCP-1: C-C motif chemokine ligand 2/monocyte chemoattractant protein 1; MMP7: matrix metallopeptidase 7; RAGE: advanced glycosylation end product-specific receptor; TIMP1: metallopeptidase inhibitor 1; IL-6: interleukin 6; IL-1β: interleukin 1 beta; TNFα: tumor necrosis factor; SERPINE1/PAI-1: serpin family E member 1/Plasminogen activator inhibitor-1

Outcome

Table E9 summarizes outcome in patients who developed versus patients who did not develop PGD at discharge. Indicators considered are the length of stay in ICU (days) and hospital (days) and the duration of mechanical ventilation (days). Gas exchange parameters ($PaCO_2$ (mmHg) and

PaO$_2$/FiO$_2$), pulmonary function tests (FEV1 (%) and FEV1/FVC (%)) and assessment of cardiovascular function (distance walked at the six minutes walk test (meters)) were recorded.

Table E9

	PGD	NO PGD	p-value
LOS ICU (days)	8 [6-12]	3 [2-5]	<0.01
LOS H (days)	30 [18-33]	16 [14-21]	0.03
Duration of mechanical ventilation (days)	5 [3-8]	1 [1-1]	<0.01
PaCO$_2$ (mmHg)	34 [31-38]	42 [36-47]	0.01
PaO$_2$/FiO$_2$	352 [324-390]	410 [364-437]	0.05
FEV1 (%)	57 [50-67]	60 [55-74]	0.57
FEV1/FVC (%)	82 [81-82]	92 [87-95]	0.02
Six minutes walk test (meters)	222 [56-322]	385 [315-443]	0.02

Table E9 – Outcome in patients who did or did not develop PGD. LOS: length of stay; ICU: intensive care unit; H: hospital; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity. Data are presented as median[IQR] and compared with Mann-Whitney test.

Main hemodynamic parameters recorded in preoperative, intra-operative and post-operative periods in patients who developed and who did not develop primary graft disfunction.

In Table E10 we compare at each timepoint data from patients who developed primary graft dysfunction and data from patients who did not develop it. After stabilization, all the hemodynamic parameters were identical in the two groups of patients, except CVP (mmHg), which was higher in patients who developed PGD, associated to an increased in heart rate (bmp) After 24 hours from the surgical procedure, patients who developed PGD presented a higher CVP (mmHg), associated to a positive 24 hours fluid balance (mL).

Table E10

		Preoperative evaluation	Intraoperative		Postoperative				
			Induction	End of surgery	0	6	24	48	72
Hearth rate (bpm)	PGD	79 [74;100]	84 [79;91]	91 [91;105]	94* [86;102]	97 [85;99]	98 [82;104]	103 [98;115]	87 [80;100]
	NO PGD	82 [77;87]	80 [65;93]	85 [83;90]	79 [74;84]	95 [83;99]	91 [86;102]	91 [84;94]	90 [81;100]
Mean arterial pressure (mmHg)	PGD	97 [90;101]	81 [73;88]	70 [68;85]	71 [66;83]	83 [79;94]	74 [72;85]	89 [86;109]	94 [88;106]
	NO PGD	88 [76;97]	73 [72;91]	72 [66;74]	80 [75;86]	81 [73;93]	80 [73;85]	85 [76;95]	88 [78;103]
Central venous pressure (mmHg)	PGD	7 [6;11]	13 [10;14]	11 [7;13]	12 [10;14]	13* [9;14]	10* [10;13]	10 [5;11]	9 [7;12]
	NO PGD	6 [4;11]	10 [7;13]	10 [9;12]	11 [9;13]	9 [8;10]	8 [7;10]	8 [5;9]	7 [5;12]
Pulmonary mean arterial pressure (mmHg)	PGD	31 [26;35]	32 [28;37]	28 [21;33]	27 [25;34]	31 [31;34]	31 [26;34]	NA	NA
	NO PGD	27 [25;36]	31 [29;33]	27 [25;29]	25 [21;31]	29 [27;31]	27 [21;30]	NA	NA
Wedge Pressure (mmHg)	PGD	12 [9;13]	15 [14;16]	NA	NA	NA	14 [13;16]	NA	NA
	NO PGD	10 [6;13]	12 [11;15]	NA	NA	NA	15 [14;19]	NA	NA
Cardiac output (L/min)	PGD	5.48 [4.95;6.44]	5.40 [4.75;6.10]	4.70 [4.03;5.30]	5.00 [4.50;5.75]	6.50 [6.30;6.80]	6.00 [4.60;6.87]	NA	NA
	NO PGD	5.16 [4.51;5.98]	4.80 [4.00;5.50]	5.80 [4.75;6.90]	6.05 [5.18;6,40]	6.10 [5.32;7.63]	6.10 [5.58;8.13]	NA	NA
Fluid balance (mL)	PGD	NA	815 [400;1090]	3305 [2002;4890]	NA	NA	722* [11;2600]	91 [-158;1304]	-200 [-1633;430]
	NO PGD	NA	950 [700;1000]	4570 [3075;7018]	NA	NA	-757 [-1230;1000]	-17 [-1332;444]	220 [-308;1038]

Table E10 - Main hemodynamic parameters recorded in preoperative, intraoperative and post-operative periods in patients who developed and who did not develop primary graft. Data are presented as median[IQR] and compared with Mann-Whitney test. NA: not available. *=p<0.05 PGD versus NO PGD.

Main gas exchange and oxygen-related parameters recorded in preoperative, intraoperative and post-operative periods in patients who developed and who did not develop primary graft disfunction.

In Table E11 we compare at each timepoint data from patients who developed primary graft dysfunction and data from patients who did not develop it. Immediately after induction of general anesthesia, before any surgical approach, patients who developed PGD presented an increased artero-venous difference in oxygen content ($mL/100mL_{blood}$). At the end of the surgical procedure, patients who developed PGD had yet an increased artero-venous difference in oxygen content ($mL/100mL_{blood}$) associated to a lower arterial pH and increased lactates.

Table E11

		Preoperative evaluation	Intraoperative		Postoperative				
			Induction	End of surgery	0	6	24	48	72
PaO₂/FiO₂	*PGD*	251 [205-343]	242 [174-277]	190* [123-292]	186* [135-335]	166* [145-196]	171* [141-184]	221* [212-235]	213* [209-275]
	NO PGD	303 [271-348]	380 [242-416]	370 [330-390]	374 [324-424]	321 [267-397]	352 [283-439]	319 [266-366]	285 [268-331]
PaCO₂ (mmHg)	*PGD*	45 [39-48]	53 [49-55]	52* [49-55]	45 [42-50]	45 [40-47]	43 [39-43]	45 [36-53]	48 [43-49]
	NO PGD	42 [38-44]	54 [46-60]	46 [37-49]	44 [38-47]	44 [39-47]	44 [41-47]	47 [40-53]	44 [36-49]
pH	*PGD*	7.43 [7.42;7.46]	7.35 [7.34;7.39]	7.28* [7.22;7.35]	7.35 [7.28;7.40]	7.40 [7.30;7.44]	7.43 [7.38;7.45]	7.43 [7.43;7.44]	7.41 [7.39;7.44]
	NO PGD	7.43 [7.41;7.45]	7.35 [7.31;7.39]	7.36 [7.31;7.39]	7.38 [7.33;7.41]	7.41 [7.38;7.42]	7.42 [7.39;7.44]	7.41 [7.36;7.43]	7.43 [7.39;7.47]
Lactates (mmol/l)	*PGD*	NA	0.70 [0.70;1.00]	2.50* [2.20;7.90]	3.30 [2.20;8.50]	2.60 [1.40;3.70]	1.50 [1.10;2.10]	1.20 [1.00;1.70]	1.00 [1.00;1.50]
	NO PGD	NA	0.60 [0.52;0.70]	1.80 [1.05;3.23]	2.30 [1.10;3.22]	1.50 [1.30;2.25]	1.10 [0.95;1.45]	1.10 [0.98;1.63]	1.10 [0.90;1.40]
SvO₂ (%)	*PGD*	NA	80.8 [76.8;82.0]	70.0 [69.0;75.0]	68.5 [62.0;70.3]	70.2 [65.4;73.3]	70.2 [63.4;73.3]	69.6 [68.5;75.4]	76.2 [65.1;81.1]
	NO PGD	NA	82.5 [78.6;85.5]	82.0 [81.0;85.0]	71.0 [67.8;73.4]	71.1 [67.3;74.2]	72.7 [67.1;76.0]	69.4 [64.8;73.0]	68.8 [65.7;74.8]
AV difference in oxygen content (mL/dL)	*PGD*	NA	3.4* [3.3;3.8]	4.1* [3.6;5.0]	5.0* [4.4;5.6]	4.3 [3.7;4.8]	4.1 [3.7;4.3]	3.5 [3.1;3.9]	3.0 [2.1;3.8]
	NO PGD	NA	2.8 [2.2;3.2]	2.4 [2.0;3.4]	4.0 [3.6;4.5]	3.9 [3.3;4.5]	3.7 [3.3;4.1]	3.7 [3.2;4.2]	3.6 [2.6;4.0]
Shunt (%)	*PGD*	NA	25.0 [24.0;29.3]	22.9 [13.8;23.3]	23.1* [19.7;27.8]	29.9* [22.5;31.9]	22.9* [19.9;28.7]	24.9* [20.1;35.4]	23.4 [18.3;38.3]
	NO PGD	NA	25.3 [20.0;31.8]	26.7 [20.7;31.6]	13.2 [11.2;17.5]	17.6 [16.6;21.6]	14.9 [8.6;19.2]	13.7 [12.0;18.5]	16.2 [12.2;31.1]

Table E11 - Main gas exchange and oxygen-related parameters recorded in preoperative, intraoperative and post-operative periods in patients who developed and who did not develop primary graft disfunction. Data are presented as median[IQR] and compared with Mann-Whitney test. NA: not available. *=p<0.05 PGD versus NO PGD.

Table E12

	Pre-operative (12 patients)	Post-operative (12 patients)	p-value
Total tissue (g)	669 [555-762]	644 [485-649]	0.11
Total gas (mL)	1058 [798-1427]	614 [364-686]	<0.01
Not-inflated tissue (%)	8 [6-13]	18 [10-26]	0.02
Poorly-inflated tissue (%)	34 [29-39]	44 [41-51]	0.03
Well-inflated tissue (%)	55 [47-64]	34 [25-45]	0.01
Over-inflated tissue (%)	0.34 [0.16-,0.64]	0.04 [0.01-0.09]	<0.01
Inhomogeneity (%)	16 [12-20]	25 [18-30]	<0.01

Table E12: Quantitative CT-scan analysis of the native not-transplanted lungs in patients who underwent single-lung transplantation, before and after surgery, with one-lung mechanical ventilation. Preoperative CT scan was available for 12 of the 14 single-lung transplantation patients. Data are median[IQR]. Mann-Whitney test.

Regressions between CT-PET and clinical data

Ex vivo lung perfusion (EVLP) was performed prior to two single lung and one double lung transplantations. At 72 hours, both patients who underwent single lung transplantation after EVLP had a grade 2 PGD, one improving from grade 3 diagnosis at 24 hours after surgery.

In the following regression, patients whose grafts underwent EVLP in the pre-operative period or their respective grafts are pictured with empty squares. As shown, the 3 patients who had reconditioned grafts had comparable clinical and imaging variables. Native not-transplanted lungs are drawn as red points in per-lung analysis.

Figure E1

A

B
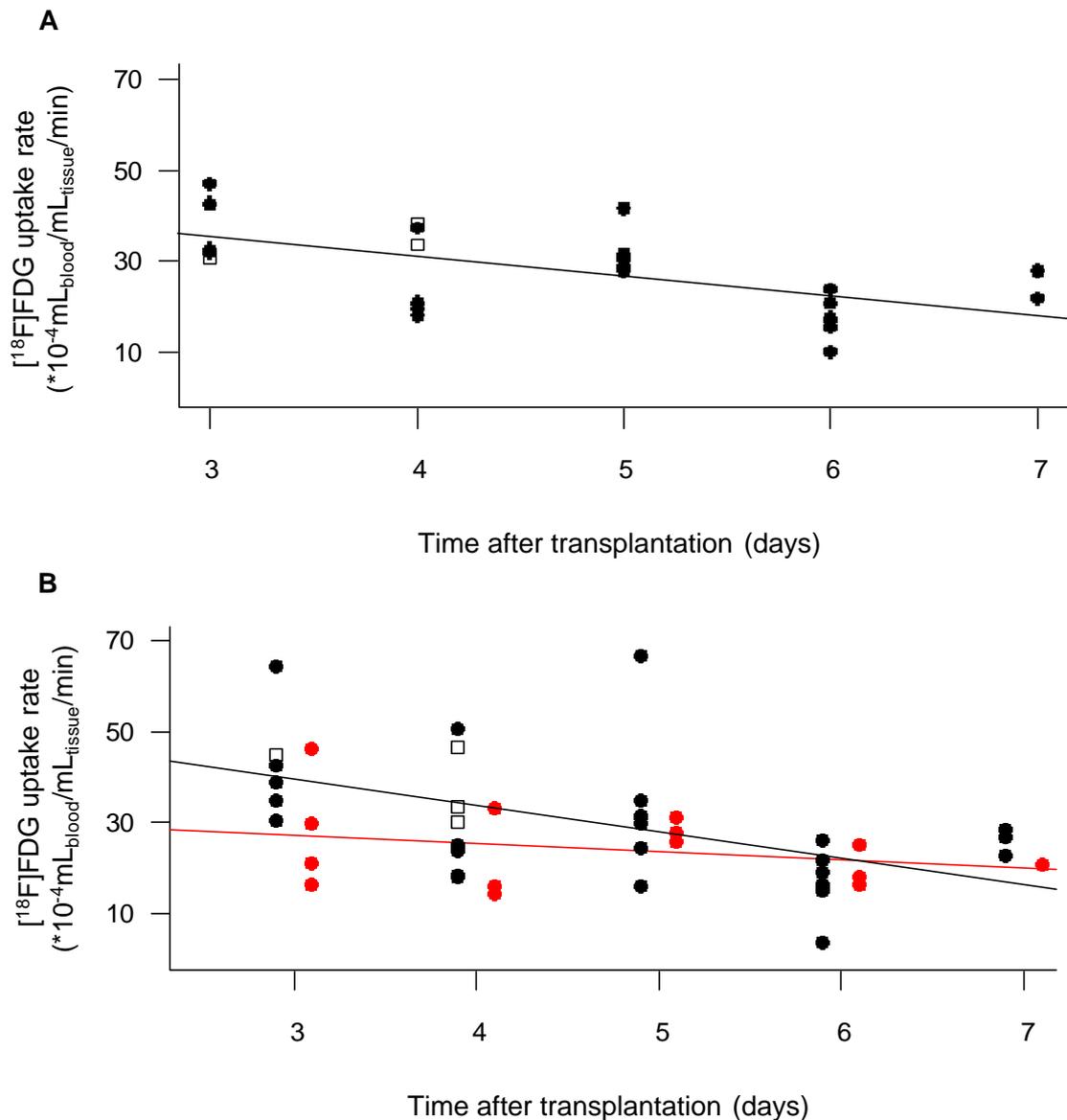
Figure E1: [^{18}F]FDG uptake rate and time after transplantation in patients, grafts and native not-transplanted lungs. Panel A presents the relationship between [^{18}F]FDG uptake rate (*10^{-4} mL$_{blood}$/mL$_{tissue}$/min) and time after transplantation (days) in patients (each point represents 1 patient). [^{18}F]FDG uptake rate (*10^{-4} mL$_{blood}$/mL$_{tissue}$/min) = 48.44 - 4.36*time after transplantation (days), R^2=0.35, p=0.002. Panel B presents the relationship between [^{18}F]FDG uptake rate (*10^{-4}mL$_{blood}$/mL$_{tissue}$/min) and time after transplantation (days) in grafts and native not-transplanted lungs (each point represents 1 lung). In grafts (black), [^{18}F]FDG uptake rate (*10^{-4}mL$_{blood}$/mL$_{tissue}$/min) = 56.79 - 5.75*time after transplantation (days), R^2=0.28, p=0.001. In native

not-transplanted lungs (red), $[^{18}F]$FDG uptake rate ($*10^{-4}mL_{blood}/mL_{tissue}/min$) = 32.86 - 1.84*time after transplantation (days), R^2=0.08, p=0.33.

Figure E2

A

B
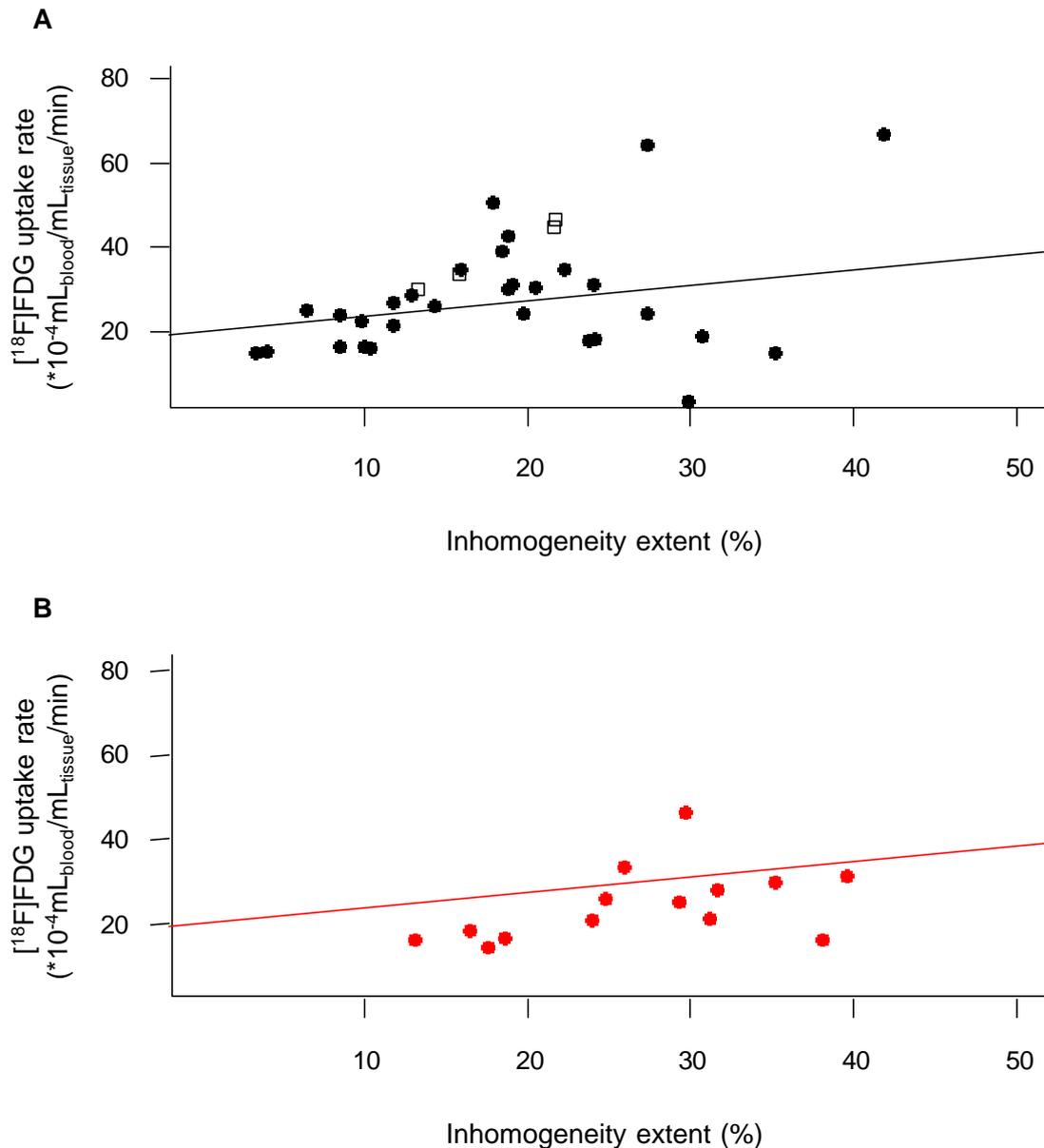
Figure E2: [^{18}F]FDG uptake rate and lung inhomogeneity extent in grafts and native not-transplanted lungs. Panel A presents the relationship between [^{18}F]FDG uptake rate ($*10^{-4}$ mL$_{blood}$/mL$_{tissue}$/min) and inhomogeneity extent (%) in grafts. [^{18}F]FDG uptake rate ($*10^{-4}$ mL$_{blood}$/mL$_{tissue}$/min) = 18.64 + 0.57*inhomogeneity extent (%), R^2=0.13, p=0.03. Panel B presents the relationship between [^{18}F]FDG uptake rate ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min) and inhomogeneity extent

(%) in native not-transplanted lungs. $[^{18}F]FDG$ uptake rate $(*10^{-4}mL_{blood}/mL_{tissue}/min) = 10.92 +$

$0.50*$inhomogeneity extent (%), $R^2=0.22$, p=0.09.

**Figure E3**

A

B
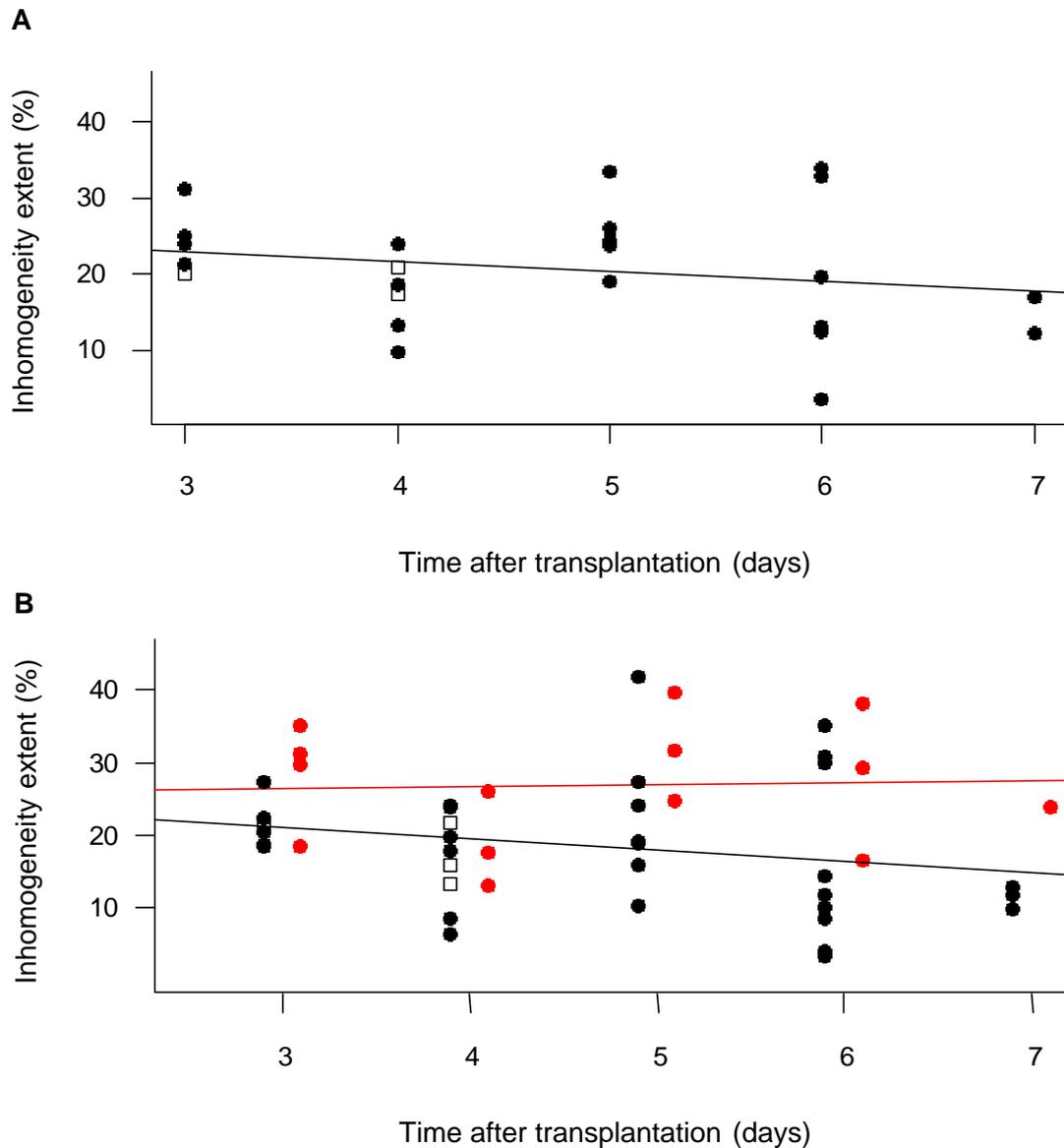
Figure E3: Inhomogeneity extent and time after transplantation in patients, grafts and native not-transplanted lungs. Panel A presents the relationship between inhomogeneity extent (%) and time after transplantation (days) in patients (each point represents 1 patient). Inhomogeneity extent (%) = 26.77 – 1.27*time after transplantation (days), R^2=0.04, p=0.32. Panel B presents the relationship between inhomogeneity extent (%) and time after transplantation (days) in grafts and native not-transplanted lungs (each point represents 1 lung). In grafts (black), Inhomogeneity extent (%) = 25.78 – 1.56*time after transplantation (days), R^2=0.05, p = 0.20. In native not-transplanted

lungs (red), Inhomogeneity extent (%) = 25.55 − 0.27*time after transplantation (days), R^2=0.002,

p=0.88.

Figure E4

A

Reactive C protein (mg/dL)

$[^{18}F]FDG$ uptake rate$(*10^{-4}mL_{blood}/mL_{tissue}/min)$

B

Reactive C protein (mg/dL)

Time after transplantation (days)
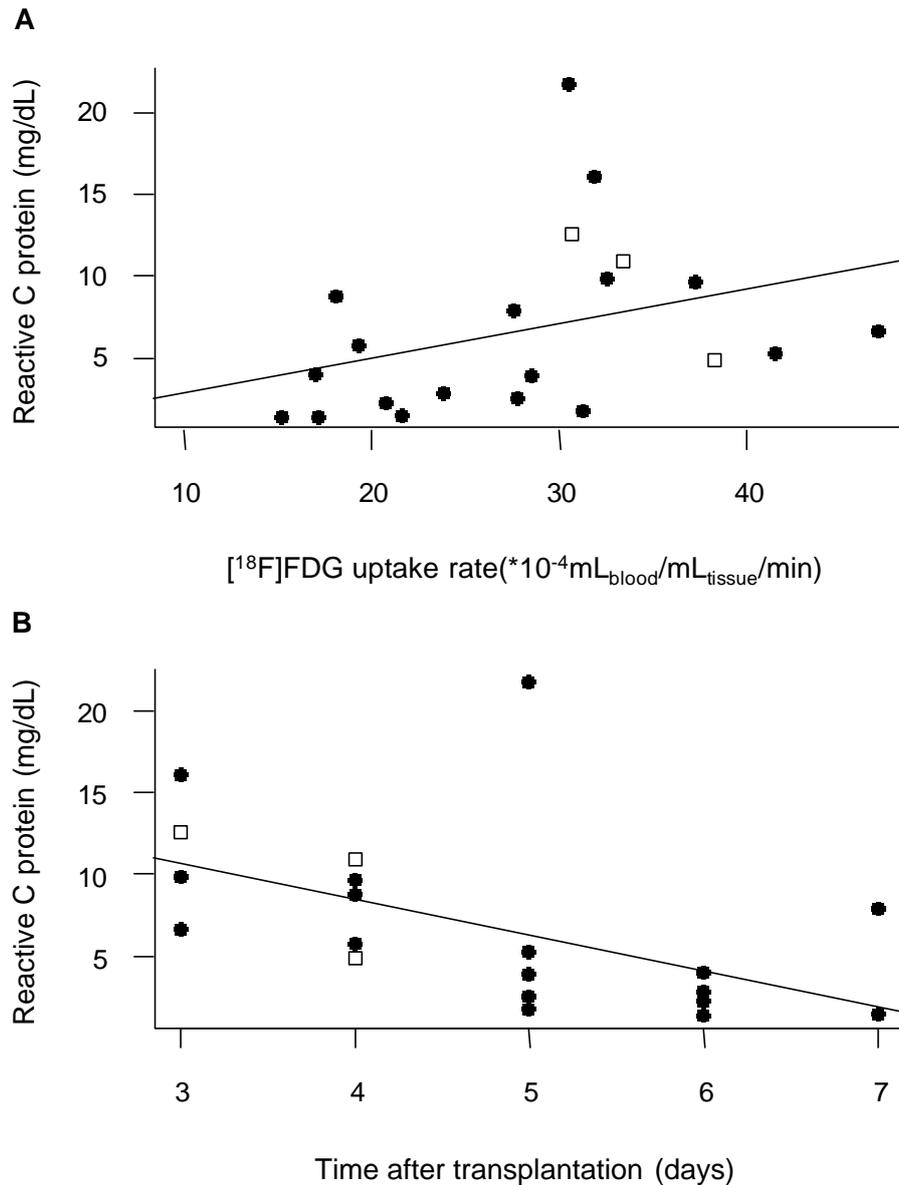
Figure E4: Reactive C protein and $[^{18}F]FDG$ uptake rate and time after transplantation. Panel A presents the relationship between Reactive C protein (mg/dL) and $[^{18}F]FDG$ uptake rate ($*10^{-4}$ $mL_{blood}/mL_{tissue}/min$). Reactive C protein (mg/dL) = 0.75 + 0.21*$[^{18}F]FDG$ uptake rate ($*10^{-4}$ $mL_{blood}/mL_{tissue}/min$), R^2=0.12, p=0.13. Panel B presents the relationship between Reactive C protein (mg/dL) and time after transplantation (days). Reactive C protein (mg/dL) = 17.25 + 2.19*time after transplantation (days), R^2=0.28, p=0.014.

Figure E5

A

B
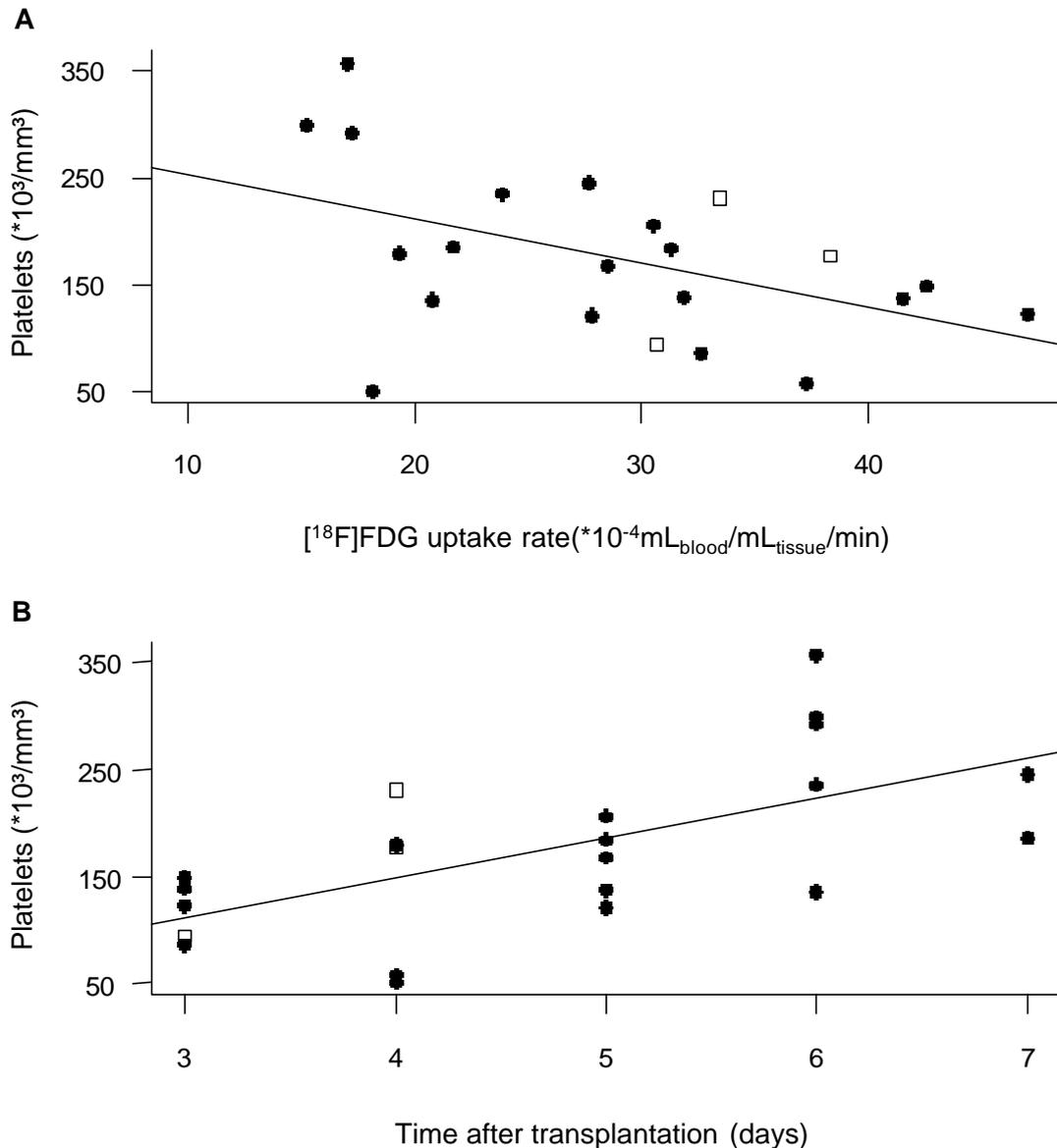
Figure E5: Platelets, [18F]FDG uptake rate and time after transplantation. Panel A presents the relationship between platelets ($10^3/mm^3$) and [18F]FDG uptake rate ($*10^{-4}mL_{blood}/mL_{tissue}/min$). Platelets ($10^3/mm^3$) = 234.9 + 2.91*[18F]FDG uptake rate ($*10^{-4}mL_{blood}/mL_{tissue}/min$), R^2=0.07, p=0.23. Panel B presents the relationship between platelets ($10^3/mm^3$) and time after transplantation (days). Platelets ($10^3/mm^3$) = -1.32 + 37.26*time after transplantation (days), R^2=0.39, p=0.002.

Figure E6

A

B
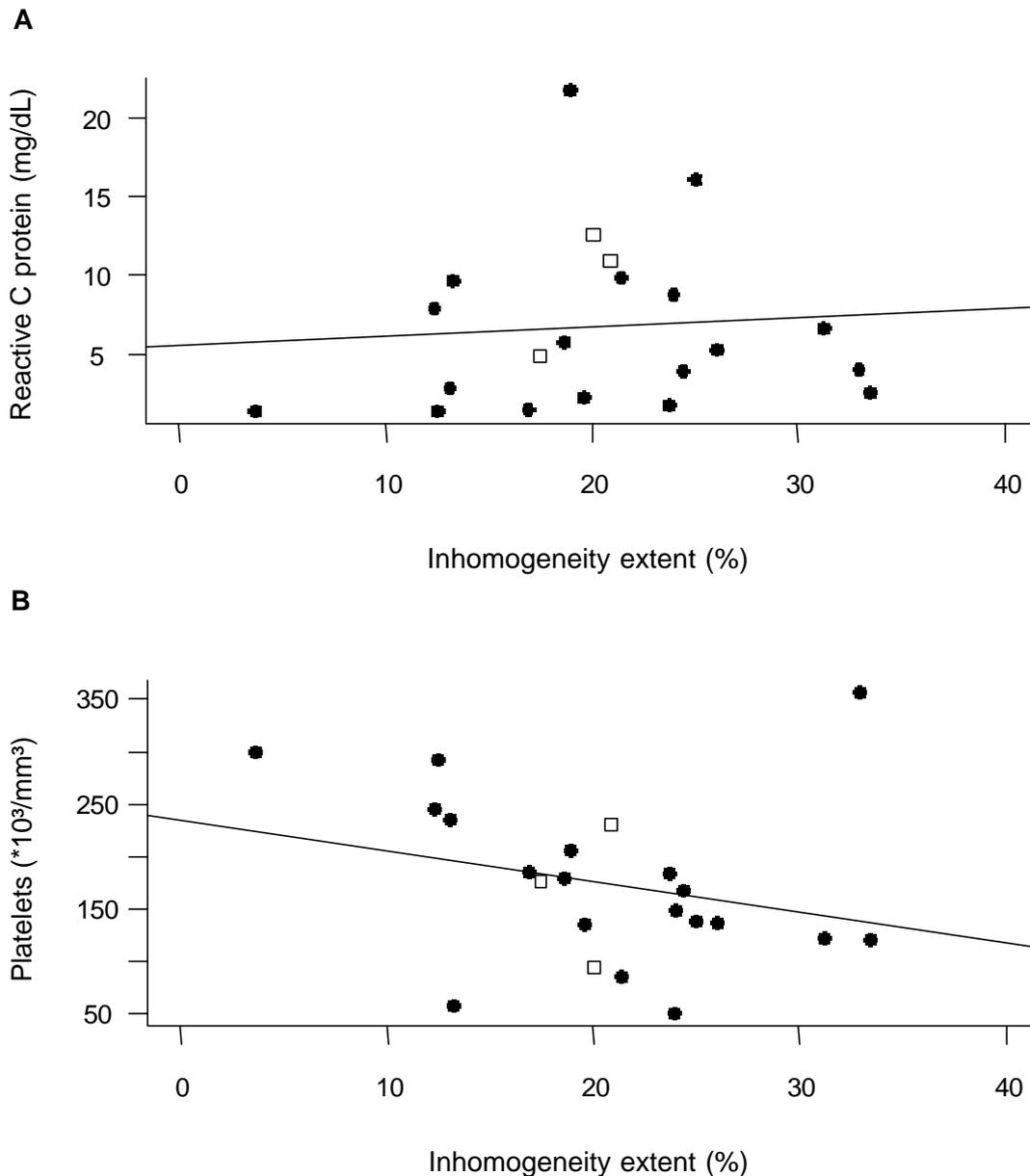
**Figure E6: Reactive C protein, platelets and inhomogeneity extent.** Panel A presents the relationship between Reactive C protein (mg/dL) and inhomogeneity extent (%). Reactive C protein (mg/dL) = 5.50 + 0.06*inhomogeneity extent (%), R^2=0.01, p=0.72. Panel B presents the relationship between platelets ($10^3/mm^3$) and inhomogeneity extent (%). Platelets ($10^3/mm^3$) = -1.32 + 37.26*inhomogeneity extent (%), R^2=0.07, p=0.23.

Figure E7

A

B

**C**
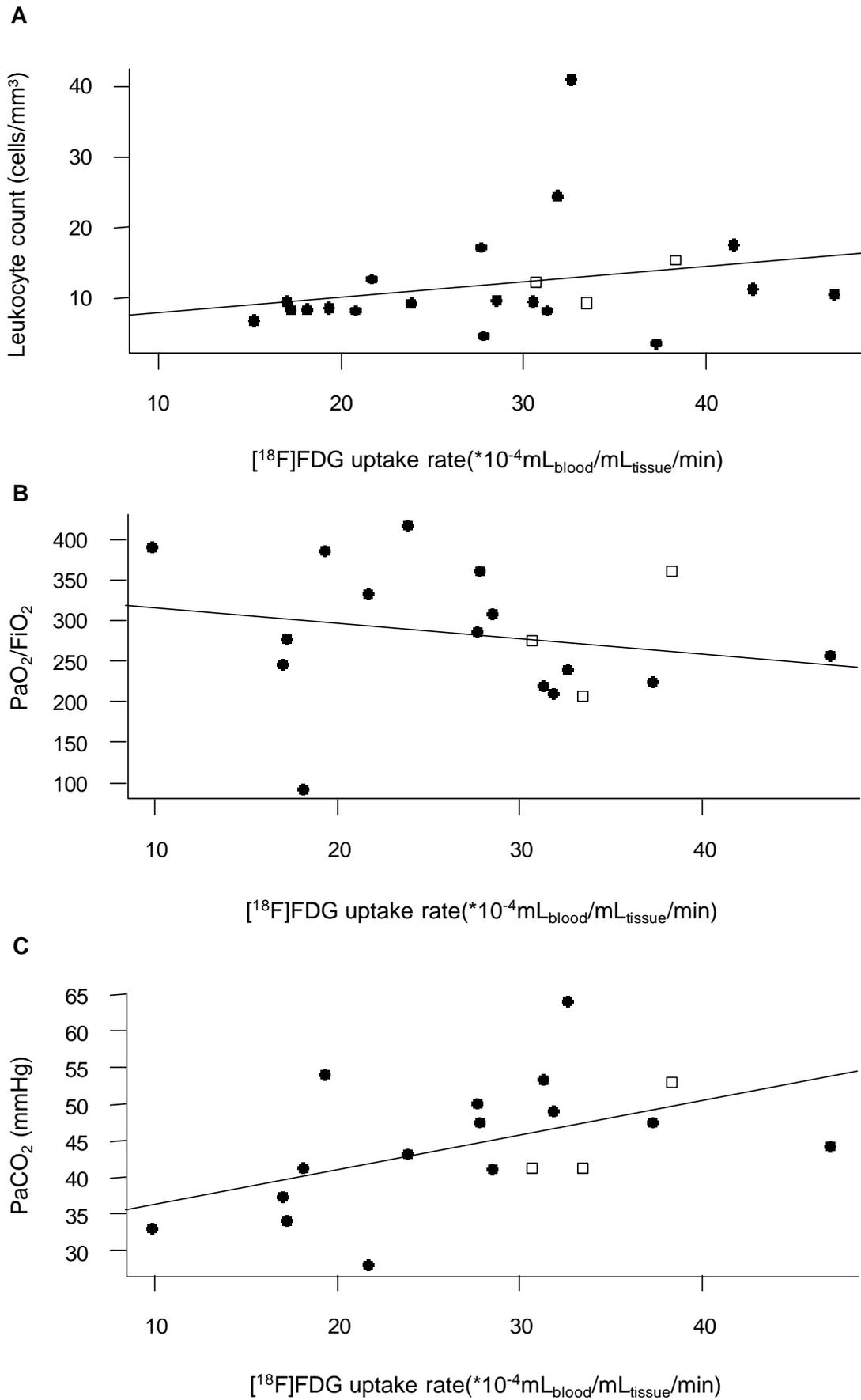
Figure E7: Leukocyte count, PaO$_2$/FiO$_2$ and PaCO$_2$ and [^{18}F]FDG uptake rate. Panel A presents the relationship between leukocyte count (cells/mm^3) and [^{18}F]FDG uptake rate ($*10^{-4}$ mL$_{blood}$/mL$_{tissue}$/min). Leukocyte count (cells/mm^3) = 5.83 + 0.21*[^{18}F]FDG uptake rate ($*10^{-4}$ mL$_{blood}$/mL$_{tissue}$/min), R^2=0.06, p=0.27. Panel B presents the relationship between PaO$_2$/FiO$_2$ and [^{18}F]FDG uptake rate ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min). PaO$_2$/FiO$_2$ = 334.6 − 1.91*[^{18}F]FDG uptake rate ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min), R^2=0.05, p=0.39. Panel C presents the relationship between PaCO$_2$ (mmHg) and [^{18}F]FDG uptake rate ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min). PaCO$_2$ (mmHg) = 31.56 − 0.47*[^{18}F]FDG uptake rate ($*10^{-4}$mL$_{blood}$/mL$_{tissue}$/min), R^2=0.25, p=0.04.

Sternum-vertebral CT-PET analysis

Sternum-vertebral CT-PET analysis was performed considering grafts from single and double lung transplantation. Tissue mass and lung density in grafts of patients who developed PGD the amount of tissue increased with an higher slope along the sternum-vertebral axis than in grafts of patients who did not develop PGD. These findings, taken together, suggested that patients who developed PGD presented some degree of pulmonary edema. The increase in [^{18}F]FDG uptake rate (*10^{-4} $mL_{blood}/mL_{tissue}/min$) we measured along the sternum-vertebral axis (see Figure 1 of the main text) could be due to the increase amount of tissue in the most dependent levels.

In the graphs below, median[IQR] of grafts of patients who developed PGD are pictured with red points/lines while median[IQR] of grafts of patients who did not are pictured in black squares/lines.

Figure E8

A

B
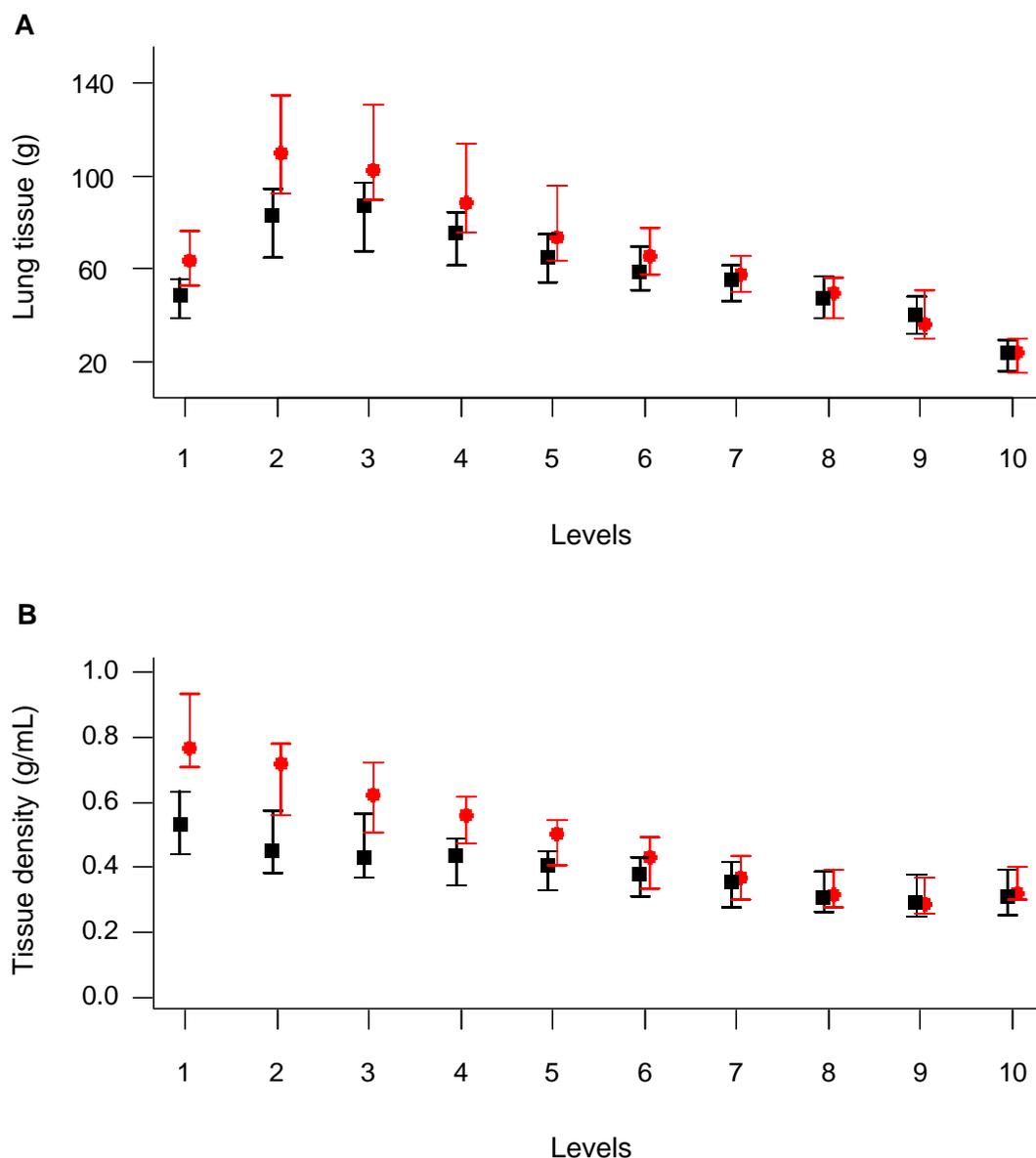
Figure E8: Tissue and density distribution in grafts of patients who developed PGD and in grafts of patients who did not. Sternum-vertebral lung tissue and tissue density were fit with linear regressions performed on all data points. The slopes were compared. Panel A shows the distribution of lung tissue (g) along sternum-vertebral axis. In grafts of patients who developed PGD, lung tissue (g)=20.97+8.3*level, R^2=0.39, p<0.0001. In grafts of patients who did not develop PGD, lung tissue (g)=29.1+5.6*level, R^2=0.39, p<0.0001. Slope in grafts of patients who developed PGD versus slope in grafts of patients who did not: p=0.004. Panel B shows the distribution of lung density (g/mL) along sternum-vertebral axis. In grafts of patients who developed PGD, lung density (g/mL)=0.31+0.025*level, R^2=0.22, p<0.0001. In grafts of patients who did not develop PGD, lung

density (g/mL)=0.031+0.039*level, R^2=0.39, p<0.0001. Slope in grafts of patients who developed

PGD versus slope in grafts of patients who did not: p=0.001. Data are presented as median[IQR].

References

1. Matute-Bello G, Liles WC, Radella F 2nd, Steinberg KP, Ruzinski JT, Jonas M, et al. Neutrophil apoptosis in the acute respiratory distress syndrome. Am J Respir Crit Care Med. 1997 Dec;156(6):1969–77.

2. Steinberg KP, Milberg JA, Martin TR, Maunder RJ, Cockrill BA, Hudson LD. Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. Am J Respir Crit Care Med. 1994 Jul;150(1):113–22.

3. Cressoni M, Chiurazzi C, Gotti M, Amini M, Brioni M, Algieri I, et al. Lung inhomogeneities and time course of ventilator-induced mechanical injuries. Anesthesiology. 2015 Sep;123(3):618–27.

4. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 1983 Mar;3(1):1–7.

5. Cressoni M, Cadringher P, Chiurazzi C, Amini M, Gallazzi E, Marino A, et al. Lung inhomogeneity in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med. 2014 Jan 15;189(2):149–58.

6. Mead J, Takishima T, Leith D. Stress distribution in lungs: a model of pulmonary elasticity. J Appl Physiol. 1970 May;28(5):596–608.

7. Haefeli-Bleuer B, Weibel ER. Morphometry of the human pulmonary acinus. Anat Rec. 1988 Apr;220(4):401–14.

8. Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant Off Publ Int Soc Heart Transplant. 2005 Oct;24(10):1454–9.

9. Willems S, Verleden SE, Vanaudenaerde BM, Wynants M, Dooms C, Yserbyt J, et al. Multiplex protein profiling of bronchoalveolar lavage in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. Ann Thorac Med. 2013 Jan;8(1):38–45.

10. Thillai M, Eberhardt C, Lewin AM, Potiphar L, Hingley-Wilson S, Sridhar S, et al. Sarcoidosis and tuberculosis cytokine profiles: indistinguishable in bronchoalveolar lavage but different in blood. PloS One. 2012;7(7):e38083.

11. Sugiyama K, Mukae H, Ishii H, Kakugawa T, Ishimoto H, Nakayama S, et al. Elevated levels of interferon gamma-inducible protein-10 and epithelial neutrophil-activating peptide-78 in patients with pulmonary sarcoidosis. Respirol Carlton Vic. 2006 Nov;11(6):708–14.

12. Huh JW, Kim DS, Oh Y-M, Shim TS, Lim CM, Lee SD, et al. Is metalloproteinase-7 specific for idiopathic pulmonary fibrosis? Chest. 2008 May;133(5):1101–6.

13. Ayhan G, Tas D, Yilmaz I, Okutan O, Demirer E, Ayten O, et al. Relation between inflammatory cytokine levels in serum and bronchoalveolar lavage fluid and gene polymorphism in young adult patients with bronchiectasis. J Thorac Dis. 2014 Jun;6(6):684–93.