Original Article

Incidence of primary graft dysfunction is higher according to the new ISHLT 2016 guidelines and correlates with clinical and molecular risk factors

Daoud Daoud[1], Lourdes Chacon Alberty[2], Qi Wei[1], Camila Hochman Mendez[2], Muhammad Hassan Masood Virk[3], Jonathan Mase[2], Peter Jindra[1], Matthew Cusick[1], Hyewon Choi[1], Natalie Debolske[1], Luiz C. Sampaio[4], Doris A. Taylor[5], Gabriel Loor[1]

[1]Michael E DeBakey Department of Surgery, Division of Cardiopulmonary Transplantation and Mechanical Circulatory Support, Baylor College of Medicine, Houston, TX, USA; [2]Department of Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA; [3]Center for Antimicrobial Resistance and Microbial Genomics (CARMiG), Department of Internal Medicine, Division of Infectious Diseases, University of Texas Health Science Centre at Houston, Houston, TX, USA; [4]Department of Advanced Cardiopulmonary Therapies and Transplantation, University of Texas Health Science Center at Houston, Houston, TX, USA; [5]RegenMedix Consulting LLC, Montgomery, AL, USA

Contributions: (I) Conception and design: L Chacon Alberty, DA Taylor, G Loor; (II) Administrative support: C Hochman Mendez, LC Sampaio, DA Taylor, G Loor; (III) Provision of study materials or patients: P Jindra, DA Taylor, G Loor; (IV) Collection and assembly of data: D Daoud, L Chacon Alberty, MHM Virk, J Mase, M Cusick, H Choi, N Debolske; (V) Data analysis and interpretation: D Daoud, L Chacon Alberty, Q Wei, MHM Virk, J Mase, P Jindra, DA Taylor, G Loor; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Gabriel Loor. Michael E DeBakey Department of Surgery, Division of Cardiopulmonary Transplantation and Mechanical Circulatory Support, Baylor College of Medicine, Houston, TX, USA. Email: Gabriel.loor@bcm.edu.

Background: Primary graft dysfunction (PGD) is the most important determinant of morbidity and mortality after lung transplantation, but its definition has evolved over the past decade. The implications of this refinement in clinical definition have not been evaluated. In this single-center study, we compared PGD incidence, risk factors, and outcomes using the 2005 and the updated-2016 International Society of Heart and Lung Transplantation guidelines for PGD grading in lung transplant patients.

Methods: In this retrospective study, we extracted data from the medical records of 127 patients who underwent lung transplantation between 1/1/2016–12/31/2018. PGD was defined as PGD3 present at 48 and/or 72 hours post-reperfusion. We used the 2005 and the updated 2016 guidelines to assess clinical risk factors, outcomes, and baseline biomarkers for PGD.

Results: On the basis of the 2016 and 2005 guidelines, we identified PGD in 37% and 26% of patients, respectively. PGD was significantly associated with extracorporeal life support, large body mass index, and restrictive lung disease using the 2016 but not the 2005 guidelines. Based on the 2016 guidelines, pretransplant levels of several biomarkers were associated with PGD; using the 2005 guidelines, only increased interleukin-2 levels were significantly associated with PGD. No preoperative biomarkers were associated with PGD using either guidelines after adjusting for clinical variables. Postoperative morbidity and 1-year mortality were similar regardless of guidelines used.

Conclusions: Our findings suggest that refinements in the PGD scoring system have improved the detection of graft injury and associated risk factors without changing its ability to predict postoperative morbidity and mortality.

Keywords: Lung transplantation; risk factors; primary graft dysfunction (PGD); cytokines; lung disease

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Introduction

Primary graft dysfunction (PGD) after lung transplantation is a primary determinant of morbidity and mortality. However, the definition of PGD has evolved over the past decade, and the analysis of the effects of these refinements on the ability to detect lung injury, risk factors, or clinical outcomes has been limited. The most widely used PGD scoring system has been the 2005 International Society of Heart and Lung Transplantation (ISHLT) guidelines based on the degree of hypoxia and pulmonary edema on chest radiograph (CXR), which have aimed to standardize PGD diagnosis. PGD is often graded on a scale of 0–3; PGD3 occurs when the ratio between partial pressure of oxygen and fraction of inspired oxygen (PaO_2:FiO_2 ratio) is <200 and edema is present on CXR, according to the 2005 guidelines (1).

Over a decade's worth of information on risk factors and clinical significance of PGD is based on the use of these guidelines (2-8). A 2016 guidelines update (9) clarified the grading of extubated patients by advocating for using the patient's estimated FiO_2 in the PaO_2:FiO_2 ratio rather than CXR findings alone, regardless of the mode of non-invasive ventilation. Additional clarifications included the use of a saturation scale for patients who may not have an arterial monitoring line, the grading of patients on extracorporeal membrane oxygenation (ECMO), and improved clarity of CXR findings (9).

The net effect of these changes on the reported incidence of PGD3 is unclear. We compared our single-center experience using the 2016 PGD scoring guidelines with results obtained using the 2005 guidelines. Specifically, we compared the incidence, clinical and biological risk factors, and outcomes associated with PGD obtained by using both scoring modalities.

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/jtd-20-3564).

Methods

Study population

We abstracted clinical data from the medical records of 127 consecutive patients who underwent a single or bilateral lung transplant between 1/1/2016–12/31/2018. In addition, we identified 60 consecutive patients (n=30 women) who underwent transplant between 3/2016–12/2017 and who had preoperative peripheral blood stored in the immunology laboratory at Baylor College of Medicine (BCM); from this cohort, we excluded patients who underwent single-lung or multiorgan transplants and those whose samples were collected >3 days before lung transplant. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the institutional ethics board at BCM (No. 00000286) with waiver of consent due to the retrospective nature of the study. The data were anonymized for the privacy of the participants.

Blood collection

The BCM immunology laboratory collects blood samples every 3–6 months from waitlisted patients for HLA profiling. Peripheral blood is collected and allowed to coagulate for 120 minutes at room temperature before centrifugation. The serum is distributed into cryotubes (without evidence of hemolysis or lipemia) and frozen immediately for storage at –80 ℃. We included patients in our study only if blood samples were collected within 3 days before transplant and had undergone <3 freeze-thaw cycles.

Measurement of circulating biomarkers

Frozen serum samples were transferred to the Texas Heart Institute Biorepository Core Laboratory for cytokine quantification via multiplex bead array technique (Bio-Plex, Bio-Rad Laboratories, Hercules, CA, USA) used according to the manufacturer's recommendations. The plates were read with the Luminex MAGPIX with a lower limit of 100 beads per sample per analyte. Data were analyzed using the Bio-plex Results Generator. We measured the concentrations of the following cytokines: basic fibroblast growth factor (FGF), vascular endothelial growth factor, platelet-derived growth factor subunit B (PDGF-BB), eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor, interferon gamma (IFN-γ), IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α and MIP-1β, RANTES, tumor necrosis factor-α, and interleukins (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, and IL-17A.

Serum concentration of the soluble form of the receptor for advanced glycation end-products (sRAGE) was assessed by enzyme-linked immunoassay (ELISA); the Human RAGE Quantikine ELISA kit (DRG00; R&D Systems, Minneapolis, MN, USA), which measures the

total pool of soluble RAGE (10), was used according to the manufacturer's instructions. Briefly, 50 µL serum was added to 100 µL of buffer, and the mixture was incubated for 2 hours and washed. Secondary antibody was added, and the mixture was incubated again for 2 hours and washed. The substrate solution was added, and the stop solution was added after a 30-minute incubation. Optical density was measured at 450 nm using the Tecan Infinite 200 PRO plate-reader.

Assays were performed in triplicate for the standard curves and in duplicate for patient samples. A coefficient of variation <20% between measurements was used as the acceptance criteria. Personnel performing assays were blinded to the clinical characteristics of the patients.

Determination of PGD grade

One expert PGD grader (DD) used the ISHLT 2005 and 2016 PGD guidelines and caveats (1,9) to determine PGD at 6, 24, 48, and 72 hours after reperfusion. PGD scores for 40 patients (160 scores) were compared to scores determined by another grader to examine inter-observer reliability. Overall agreement between graders was 87.5% (correlation coefficient, 0.9; P<0.0001). We herein refer to PGD as the presence of PGD3 at 48 and/or 72 hours after reperfusion. For extubated patients who had a set FiO_2 programmed on a face mask or continuous positive airway pressure or bilevel positive airway pressure device, the standard PaO_2:FiO_2 ratio was used in both scoring guidelines if the FiO_2 was >30%. For extubated patients on a nasal cannula, the PGD grade was 0 or 1 depending on the presence of edema on CXR according to the 2005 guidelines. For patients who were extubated and on nasal cannula, the estimated FiO2 was used according to the 2016 guidelines if the FiO_2 was estimated as >30%. We estimated the FiO_2 as follows: 2L =28%, 3L =32%, 4L =36%, 5L =40%, 6L =44%, etc. For extubated patients who did not have an arterial blood gas measurement and who had edema on CXR, the PGD grade was calculated according to the PaO_2:FiO_2 ratio using a saturation scale as per the 2016 guidelines (9). When such patients were graded according to the 2005 guidelines, a saturation scale was not used, but rather PGD was graded as 0 or 1, depending on the edema status on CXR.

Measurement of clinical outcomes

Clinical data on mortality, length of stay, ventilator requirement, and end-organ function were abstracted from patients' records to our lung transplant database. To ensure consistency, we compared the abstracted data with data from UNOS regulatory submissions.

Statistical analysis

The Fisher exact test was used to compare categorical variables for recipient and donor demographics, donor data, operative variables, and outcomes. Normality was evaluated using the Shapiro-Wilk test. For normally distributed data, statistical assessments were performed using a 2-tailed Student *t* test. Nonparametric comparisons were done by Mann Whitney U and Wilcoxon tests. We used univariable analysis and multivariable logistic regression to estimate the odds ratio for the relationship between clinical risk factors, biomarker levels, PGD development, and mortality. Multivariable analysis was performed with adjustment for clinical factors [age, body mass index (BMI), use of extracorporeal life support (ECLS), presence of pulmonary hypertension, and primary diagnosis]. Stepwise regression and backward regression were used to select the final multivariable regression model. We used the receiver operating characteristics (ROC) of biomarker levels to assess how well the biomarker levels predicted PGD. Differences in progression-free survival curves were estimated using the Kaplan-Meier method, and the absence or presence of mortality was evaluated by log-rank test. P values were based on a 2-sided hypothesis. P<0.05 was considered statistically significant. SAS 9.4 was used for statistical analysis, and plots were performed using GraphPad Prism 8.

Results

Clinical risk factors for PGD

We included 127 patients who met the inclusion criteria and had complete clinical data for analysis. Using the 2016 scoring guideline, we identified 47 (37%) patients with PGD3 at 48–72 hours after reperfusion. Patients in the PGD group had a significantly larger BMI than those without PGD (*Table 1*). The percentage of patients with restrictive lung disease was significantly higher in the PGD group, whereas the percentage of patients with cystic fibrosis was lower. ECLS with either ECMO or cardiopulmonary bypass was used in 89% of transplant patients and was more common in PGD cases. Based on multivariate analysis, BMI, restrictive lung disease, and ECLS use were associated with PGD, whereas diagnosis of

Table 1 Demographics and risk factors for patients without and with PGD using 2005 and 2016 guidelines

Variable	Total	2005 Guidelines			2016 Guidelines		
		Non-PGD	PGD	P value	Non-PGD	PGD	P value
Number (%)	127 (100.0)	94 (74.02)	33 (25.98)		80 (63.0)	47 (37.0)	
Recipient characteristics							
Women	48 (37.80)	38 (40.43)	10 (30.30)	0.4043	32 (40.00)	16 (34.04)	0.504
Age	52.6±16.3	51.96 ±16.52	54.45±15.85	0.4518	51.34±17.1	54.77±14.9	0.255
BMI (kg/m^2)	24.95±5.35	24.53±5.38	26.15±5.15	0.1346	24.02±5.44	26.52±4.85	0.0104
Blood type				0.848			0.763
A	43 (33.86)	30 (31.91)	13 (39.39)		25 (31.25)	18 (38.30)	
B	15 (11.81)	11 (11.70)	4 (12.12)		11 (13.75)	4 (8.51)	
AB	7 (5.51)	5 (5.32)	2 (6.06)		5 (6.25)	2 (4.26)	
O	62 (48.82)	48 (51.06)	14 (42.42)		39 (48.75)	23 (48.94)	
Rh group negative	12 (9.45)	8 (8.51)	4 (12.12)	0.5081	6 (7.50)	6 (12.77)	0.358
Primary disease				0.0664			0.0448
Restrictive lung disease	68 (53.54)	44 (46.81)	24 (72.73)		37 (46.25)	31 (65.96)	
Cystic fibrosis	27 (21.26)	24 (25.53)	3 (9.09)		23 (28.75)	4 (8.51)	
COPD	23 (18.11)	20 (21.28)	3 (9.09)		15 (18.75)	8 (17.02)	
Other	7 (5.51)	5 (5.32)	2 (6.06)		4 (5.00)	3 (6.38)	
Pulmonary vascular disease	2 (1.57)	1 (1.06)	1 (3.03)		1 (1.25)	1 (2.13)	
Primary disease				0.0144			0.0316
Restrictive lung disease	68 (53.54)	44 (46.81)	24 (72.72)		37 (46.25)	31 (65.96)	
Non-restrictive lung diseases	59 (46.46)	50 (53.19)	9 (27.27)		43 (53.75)	16 (34.04)	
Diabetes	35 (27.56)	24 (25.53)	11 (33.33)	0.4972	20 (25)	15 (31.91)	0.400
Hypertension	59 (46.46)	42 (44.68)	17 (51.52)	0.5465	33 (41.25)	26 (55.32)	0.125
History of smoking*	65 (51.18)	49 (52.13)	16 (48.48)	0.84	40 (50.00)	25 (53.19)	0.728
Lung allocation score	43.02±11.45	42.21 ±10.30	45.34±14.17	0.1777	42.42±10.42	44.06±12.86	0.439
Baseline creatinine (mg/dL)	0.89±0.45	0.90±0.52	0.86±0.20	0.6178	0.9±0.55	0.87±0.2	0.707
Condition at time of transplant				0.668			1.00
Not hospitalized	113 (88.98)	85 (90.43)	28 (84.85)		71 (88.75)	42 (89.36)	
Hospitalized (not in ICU)	6 (4.72)	4 (4.26)	2 (6.06)		4 (5.00)	2 (4.26)	
In ICU	8 (6.30)	5 (5.32)	3 (9.09)		5 (6.25)	3 (6.38)	
Life support before LTx	8 (6.30)	4 (4.26)	4 (12.12)	0.2038	4 (5.00)	4 (8.51)	0.467
Pre-operative ECMO	2 (1.57)	1 (1.06)	1 (3.03)	0.4537	1 (1.25)	1 (2.13)	1.00
Pulmonary hypertension	99 (77.95)	76 (80.85)	23 (69.70)	0.2233	63 (78.75)	36 (76.60)	0.826
Prior thoracic surgery (non-transplant)	16 (12.60)	10 (10.64)	6 (18.18)	0.3589	7 (8.75)	9 (19.15)	0.089

Table 1 (*continued*)

Table 1 (*continued*)

Variable	Total	2005 Guidelines			2016 Guidelines		
		Non-PGD	PGD	P value	Non-PGD	PGD	P value
Prior cardiac surgery	4 (3.15)	4 (4.26)	0 (0.0)	0.572	3 (3.75)	1 (2.13)	1.00
Prior pleurodesis	7 (5.51)	4 (4.26)	3 (9.09)	0.375	4 (5.00)	3 (6.38)	0.709
Prior lung transplant	3 (2.36)	2 (2.13)	1 (3.03)	1.000	1 (1.25)	2 (4.26)	0.554
Donor characteristics							
Age (years)	35.07±12.2	35.56 ±11.99	33.67±12.88	0.4444	35.1±12.24	35.02±12.26	0.972
Extended criteria donor[†]	45 (35.43)	29 (30.85)	16 (48.48)	0.068	25 (31.25)	20 (42.55)	0.198
Smoker ever (n=126)[‡]	67 (53.17)	48 (51.61)	19 (57.58)	0.6852	41 (51.90)	26 (55.3)	0.71
Smoker >20 PYH (n=126)[§]	7 (5.56)	6 (6.45)	1 (3.03)	0.6757	6 (7.59)	1 (2.13)	0.2558
Sex mismatch	39 (30.71)	27 (28.72)	12 (36.36)	0.549	22 (27.50)	17 (36.17)	0.188
Sex mismatch, female-male	20 (15.75)	11 (11.70)	9 (27.27)	0.0665	9 (11.25)	11 (23.40)	0.2003
Donor after cardiac death	11 (8.66)	5 (5.32)	6 (18.18)	0.0341	4 (5.00)	7 (14.89)	0.0980
Ex vivo lung perfusion[¶]	10 (7.87)	7 (7.45)	3 (9.09)	0.7189	6 (7.50)	4 (8.51)	1.00
Operative characteristics							
Type of transplant				0.825			0.941
Bilateral	109 (85.83)	80 (85.10)	29 (87.87)		68 (85.00)	41 (87.23)	
Single	15 (11.81)	12 (12.77)	3 (9.09)		10 (12.50)	5 (10.64)	
Multi-organ	3 (2.36)	2 (2.13)	1 (3.03)		2 (2.5)	1 (2.13)	
Type of intraoperative support				0.0201			0.017
Off-pump	14 (11.02)	14 (14.89)	0 (0.0)		13 (16.25)	1 (2.13)	
ECLS	113 (88.98)	80 (85.11)	33 (100.0)		67 (83.75)	46 (97.87)	
Type of ECLS				1.00			0.790
ECMO	17 (15.04)	12 (15)	5 (15.15)		11 (16.42)	6 (13.04)	
Cardiopulmonary bypass	96 (84.95)	68 (85)	28 (84.84)		56 (83.58)	40 (86.96)	
Total ischemic time (min)	323±120.53	315.31±120.84	346.30±118.45	0.205	317.73±120.15	332.96±121.86	0.494

Values are n (%) or mean ± SD. *, n=125 + 2 patients' smokeless tobacco (snuff); [†], one or more of the following: age >55 years, anticipated ischemia >6 hours, DCD, PaO_2/FiO_2 <300, donor is >20PYH smoker; [‡], n=126 (one unknown donor smoking history); [§], n=126 (one unknown donor smoking history); [¶], using portable EVLP system. Bilateral, double lung transplant; COPD, chronic obstructive pulmonary disease; ECLS, extracorporeal lung support; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; Multi-organ, double lung and additional organs; SD, standard deviation; Single, single lung transplant; 20PYH, 20 pack-year smoking history.

cystic fibrosis was associated with no PGD.

When grading the 127 patients by using the 2005 guidelines, 14 patients were downgraded from PGD3 to PGD1. Thus, only 33 (26%) patients met the definition for PGD (PGD3 at 48 or 72 hours) (*Table 2*). The 14 patients who were downgraded were extubated, on nasal-cannula, and received a grade of 1 due to edema on CXR. Patients with 2005 guideline-defined PGD had greater restrictive lung disease and a higher use of donor after cardiac death organs and intraoperative ECLS than those without PGD, although no factors were significant after adjusting for covariables.

The differences in the incidence of PGD3 at individual time points using the 2016 versus 2005 definition were as follows: 45.6% versus 45.6% at 6 hours (P=1), 33.9% versus 26.8% at 24 hours (P=0.22), 33.1% versus 23.6%

Table 2 Outcomes in patients without and with PGD using 2005 and 2016 guidelines

Variable	Total	2005 Guidelines			2016 Guidelines		
		Non-PGD	PGD	P value	Non-PGD	PGD	P value
Number (%)	127 (100.0)	94 (74.02)	33 (25.98)	–	80 (63.0)	47 (37.0)	–
Postoperative length of stay (days)	29.37±32.90	23.43 ±27.42	46.30±40.96	0.0005	23.86±29.22	38.75±36.83	0.013
≤21	73 (57.48)	62 (65.96)	11 (33.33)	0.0001	52 (65.00)	21 (44.68)	0.002
22–30	20 (15.75)	16 (17.02)	4 (12.12)		15 (18.75)	5 (10.64)	
>30	34 (26.77)	16 (17.02)	18 (54.55)		13 (16.25)	21 (44.68)	
ICU length of stay (days)	18.75 ±26.74	12.22±18.85	37.33±36.07	0.0001	12.31±19.72	29.7±33.1	0.0003
Peak creatinine within 48 hours (mg/dL)	1.10±0.53	1.05±0.50	1.22±0.59	0.1108	1.08±0.53	1.14±0.51	0.536
Peak creatinine value ≥1.5 mg/dL	16 (12.60)	8 (8.51)	8 (24.24)	0.0304	8 (10.00)	8 (17.02)	0.249
Max creatinine (mg/dL)	1.55±1.17	1.40±1.04	1.96±1.40	0.0179	1.39±1.04	1.82±1.31	0.0464
Max creatinine ≥1.5 mg/dL	41 (32.28)	23 (24.47)	18 (54.55)	0.0023	20 (25.00)	21 (44.68)	0.022
Discharge creatinine (mg/dL)	0.92±0.66	0.87±0.44	1.04±1.05	0.2005	0.89±0.47	0.98±0.88	0.455
Peak lactate within 72 hours (mg/dL) (n=115)	7.38±3.80	6.71±3.69	9.02±3.64	0.0028	6.95±3.75	8.02±3.83	0.137
Postoperative ECMO	18 (14.17)	3 (3.19)	15 (45.45)	<0.0000	3 (3.75)	15 (31.91)	<0.0001
Mechanical ventilation ≥48 hours	54 (42.52)	25 (26.60)	29 (87.88)	<0.0000	22 (27.50)	32 (68.09)	<0.0001
Mechanical ventilation ≥5 days	34 (26.77)	12 (12.77)	22 (66.67)	<0.0000	11 (13.75)	23 (48.94)	<0.0001
Reintubated	33 (25.98)	22 (23.40)	11 (33.33)	0.3559	20 (25.00)	13 (27.66)	0.741
Tracheostomy	31 (24.41)	13 (13.83)	18 (54.55)	<0.0000	12 (15.00)	19 (40.43)	0.0013
Airway dehiscence	4 (3.15)	1 (1.06)	3 (9.09)	0.0563	1 (1.25)	3 (6.38)	0.1428
Dialysis	19 (14.96)	7 (7.45)	12 (36.36)	0.0002	7 (8.75)	12 (25.53)	0.0105
Discharge disposition				0.00035			0.0097
Home	95 (74.80)	77 (81.91)	18 (54.55)		66 (82.50)	29 (61.70)	
Rehabilitation facility	23 (18.11)	15 (15.96)	8 (24.24)		12 (15.00)	11 (23.40)	
Death	9 (7.09)	2 (2.13)	7 (21.21)		2 (2.50)	7 (14.89)	
Readmission within 1 year (n=118)		92	26	0.258	78	40	0.263
Yes	103 (87.29)	82 (89.13)	21 (80.77)		70 (89.74)	33 (82.50)	
No	15 (12.71)	10 (10.87)	5 (19.23)		8 (10.26)	7 (17.50)	
ICU readmission within 1 year (n=118)		92	26	0.135	78	40	0.316
Yes	40 (33.90)	28 (30.43)	12 (46.15)		24 (30.77)	16 (40.00)	
90-day mortality	5 (1.57)	1 (1.06)	4 (12.12)	0.0161	1 (1.25)	4 (8.51)	0.0622
1-year mortality	16 (12.60)	5 (5.32)	11 (33.33)	0.0001	4 (5.00)	12 (25.53)	0.001

Values are n (%) or mean ± SD. ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

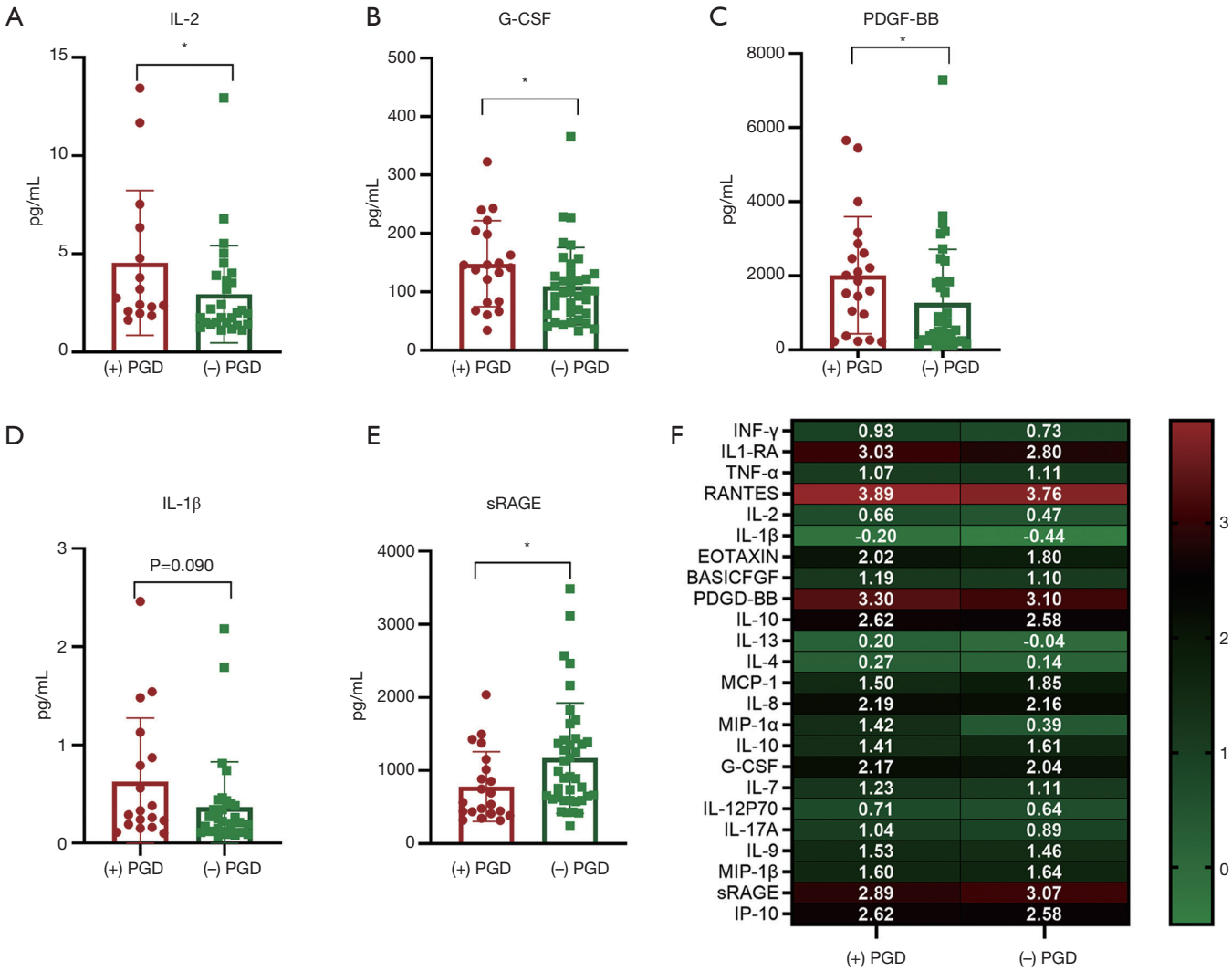

Figure 1 Comparison of baseline pro-inflammatory cytokines between patients without (–) and with (+) PGD using the 2016 scoring guidelines. (A) IL-2, (B) G-CSF, (D) PDGF-BB, and (E), IL-1β were elevated in peripheral blood at baseline and (C) sRAGE was decreased in patients who developed PGD. (F) Heat map showing that the log10 mean concentration of cytokines did not differ in patients with (+) and without (–) PGD. Each symbol represents the mean of an experiment run in duplicate for every patient. *, P<0.05. PGD, primary graft dysfunction; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; PDGF, platelet-derived growth factor.

at 48 hours (P=0.09), and 30.7% versus 22.1% at 72 hours (P=0.12), respectively. Moreover, the incidence of PGD was 20% in single lung transplants and 26.8% in double lung transplants, using the 2005 definition, and 33.3% versus 37.5%, respectively, using the 2016 definition.

Biomarkers correlated with PGD

We evaluated baseline serum biomarker concentrations in 60 patients and assessed differences between PGD and non-PGD patients scored with the 2005 and 2016 ISHLT guidelines (Table S1). Clinical characteristics were representative of the larger cohort including more patients with restrictive lung disease and a larger BMI in the PGD group (Table S2).

Using the 2016 ISHLT guidelines, the average levels of IL-2, G-CSF, and PDGF-BB (*Figure 1A,B,C*) were significantly higher in the PGD group than in the non-PGD group. A numerical increase without statistical significance was observed in IL-1β (*Figure 1D*). sRAGE

levels (*Figure 1E*) were higher in the non-PGD group. The log10 mean concentration of various cytokines (*Figure 1F*) showed greater inflammation in PGD samples as depicted by the darker shades (IFN-γ, IL-1RA, RANTES, EOTAXIN, IP-10, MIP-1A, IL-7), but no significant differences were found between the two groups. Area under the curve analyses for baseline biomarkers associated with PGD using the 2016 guidelines showed that IL-2 had the best predictive capacity for PGD, followed by sRAGE (*Figure 2*). A multivariate logistic regression analysis using 5 biomarkers (IL-2, G-CSF, PDFG-BB, IL-1β, and sRAGE), recipient age, BMI, and diagnosis of restrictive lung disease showed that BMI was the only risk factor significantly associated with PGD. Finally, multivariate logistic regression analysis using only the above-mentioned 5 biomarkers and no clinical factors showed that IL-2 and sRAGE were significantly associated with PGD (P=0.036 and P=0.028, respectively).

Using the 2005 scoring guidelines, only IL-2 was significantly different at baseline (6.65 *vs.* 3.0 pg/mL, P=0.048). Higher G-CSF, PDGF-B, and lower sRAGE levels supported the observations in the 2016 cohort, but the differences did not reach significance (Table S1). In the 2005 cohort, multivariate logistic regression analysis using 5 biomarkers (L-2, G-CSF, PDFG-BB, IL-1β, and sRAGE), recipient age, BMI, and diagnosis of restrictive lung disease did not show any correlation between baseline biomarkers or clinical risk factors and PGD.

Biomarkers correlated with clinical risk factors

In addition to exploring differences in baseline biomarkers in patients with PGD, we examined the association between these biomarkers and known PGD risk factors. Using the 2016 scoring guidelines, we analyzed the association between preoperative biomarkers and clinical risk factors for PGD. *Figure 3* summarizes the difference between biomarker levels in patients with restrictive lung disease, which showed a higher incidence in patients with PGD. sRAGE levels were significantly lower in patients with restrictive lung disease than in all other patients (766.04 *vs.* 1,399.71 pg/mL; P=0.013) but were significantly higher in patients with cystic fibrosis than in patients with other diseases (1,954.20 *vs.* 827.48 pg/mL; P<0.001) (*Figure 4*). Logistic regression showed that sRAGE was associated with restrictive lung disease. We compared biomarker levels in patients with a BMI < or >25 kg/m^2 (*Figure 5*). sRAGE levels were lower in patients with a higher BMI (740.80 *vs.* 1,290.64 pg/mL; P=0.008). Patients with a higher BMI had significantly greater levels of IL-2 (5.70 *vs.* 2.42 pg/mL; P=0.004). A linear regression model with stepwise deletion (data not shown) demonstrated that IL-2 was associated with BMI and that sRAGE levels had a significant negative correlation with age (*Figure 6A*) and BMI (*Figure 6B*). No significant correlation was found between other cytokines levels and BMI or age. Patients with lower BMI, who were younger with cystic fibrosis, and who had higher sRAGE levels appeared least likely to develop PGD (Figure S1).

Clinical outcomes correlated with PGD

No differences were seen in outcomes when comparing assessments using the two guidelines (*Table 2*). In both guidelines, multiple adverse outcomes were associated with PGD, including extended hospital and ICU stays. The use of dialysis was significantly higher in the PGD group, as were maximum creatinine levels, postoperative ECMO use, prolonged ventilation times (>5 days), and tracheostomy. Furthermore, the PGD group had lower 1-year survival rates using both guidelines (*Figure 7A,B*); 25.53% in the PGD group and 5.0% of non-PGD patients died at 1 year using the 2016 PGD definition (P=0.001). Using the 2005 PGD guidelines, 33.33% in the PGD group and 5.32% of the non-PGD patients died at 1 year (P=0.0001) (*Table 2*).

We assessed the effect of PGD on mortality by using a multiple regression model adjusting for recipient age, sex, BMI, primary disease, lung allocation score, hypertension, donor age, extended criteria donor, donor smoker, type of transplant, use of ECLS, and total ischemic time. PGD was independently associated with 1-year mortality with the use of both the 2016 [odds ratio 10.7; 95% CI (2.1, 55.2)] and the 2005 guidelines [15.2; 95% CI (2.5, 93.6)]. This association was confirmed in a stepwise selection model.

In Table S3, clinical outcomes for patients designated as having PGD according to both 2005 and 2016 guidelines (n=33) were compared to outcomes in patients who were downgraded to PGD 1 using the 2005 guidelines (n=14). The latter were designated as PGD (PGD3 at 48–72 hours) when using the 2016 guidelines due to strict adherence to saturation ratios and caveats for extubated patients. There were no differences in clinical characteristics between these groups (Table S3). Although the numbers were low, patients who were downgraded in the 2005 guidelines (n=14) had better clinical outcomes and resembled non-PGD patients. They had a significantly reduced length of stay (mean, 20 *vs.* 46 days, P=0.029, unadjusted), decreased peak lactate

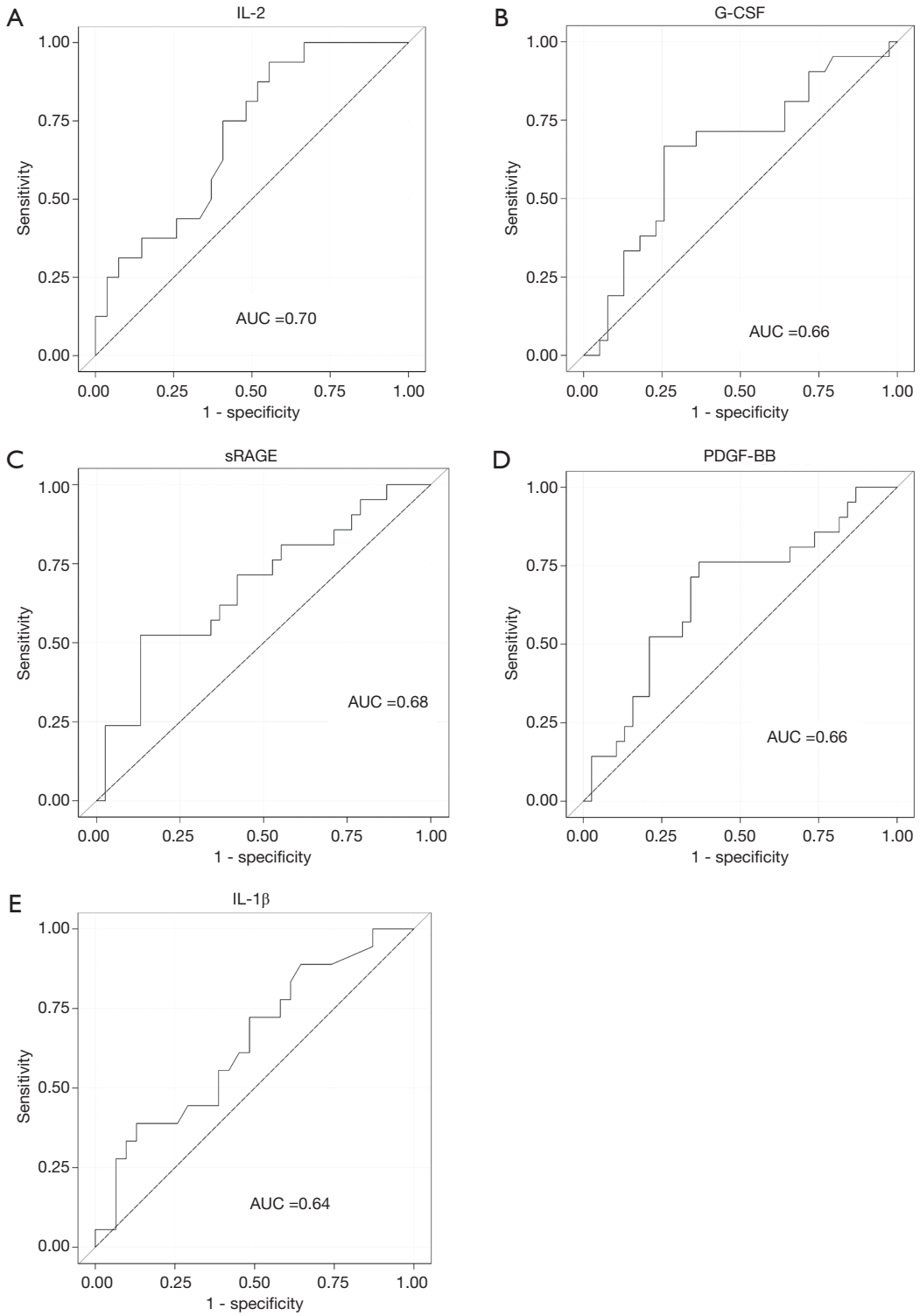

Figure 2 Receiver operating characteristics for baseline cytokines associated with PGD using the 2016 updated guidelines. (A) IL-2, (B) G-CSF, (C) sRAGE, (D) PDGF-BB, and (E) IL-1β. AUC, area under the curve; PGD, primary graft dysfunction; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; PDGF, platelet-derived growth factor.

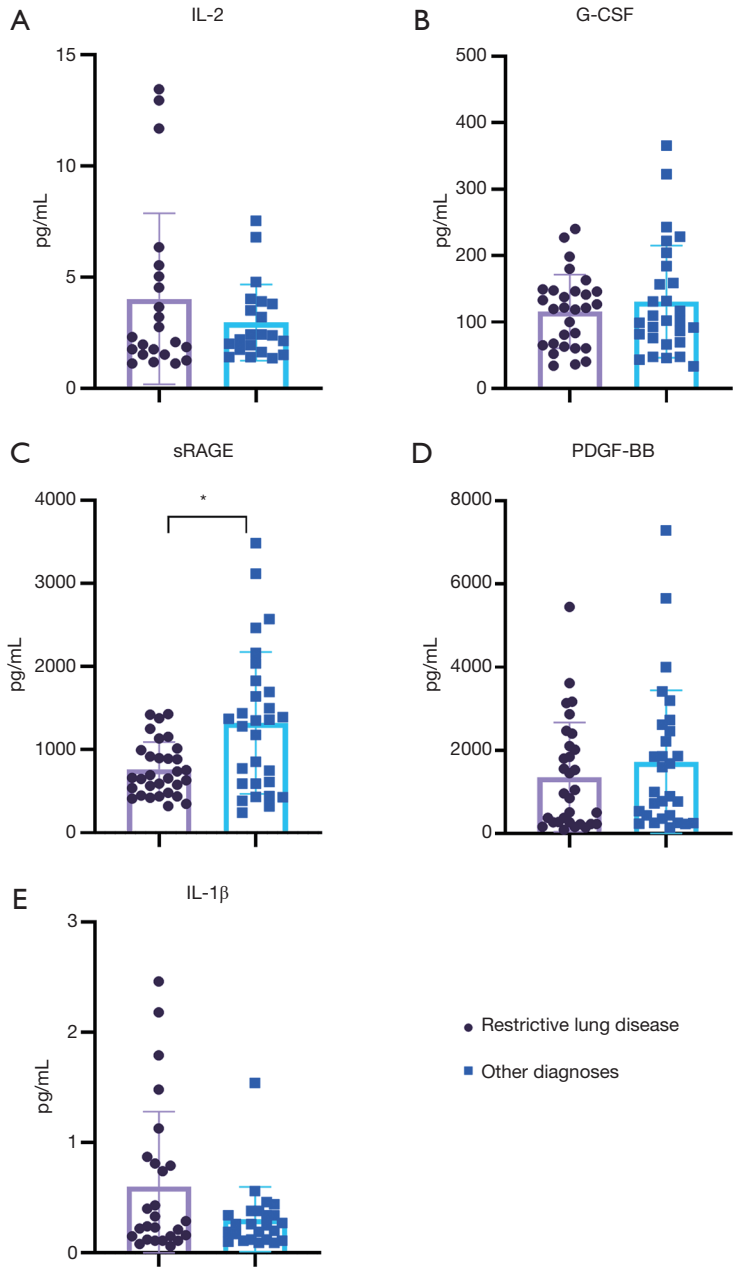

Figure 3 Bar plots of serum cytokines in patients diagnosed with restrictive lung disease versus all other baseline diagnoses. (A) IL-2, (B) G-CSF, (C) sRAGE, (D) PDGF-BB, and (E) IL-1β. *, P<0.05. IL, interleukin; G-CSF, granulocyte colony-stimulating factor; PDGF, platelet-derived growth factor.

levels (mean, 5.5 *vs.* 9 mg/dL, P=0.004, unadjusted), no postoperative ECMO, and lower rates of prolonged ventilation, tracheostomy use (7% *vs.* 55%, P=0.003), and dialysis. Downgraded patients had a numerically lower but non-significant reduction in mortality (1-year mortality, 7% *vs.* 33%, P=0.08, unadjusted).

Discussion

We examined clinical and biological risk factors and clinical outcomes associated with PGD classified according to the 2005 and the updated 2016 ISHLT guidelines for PGD at a single center. We believe our study will help

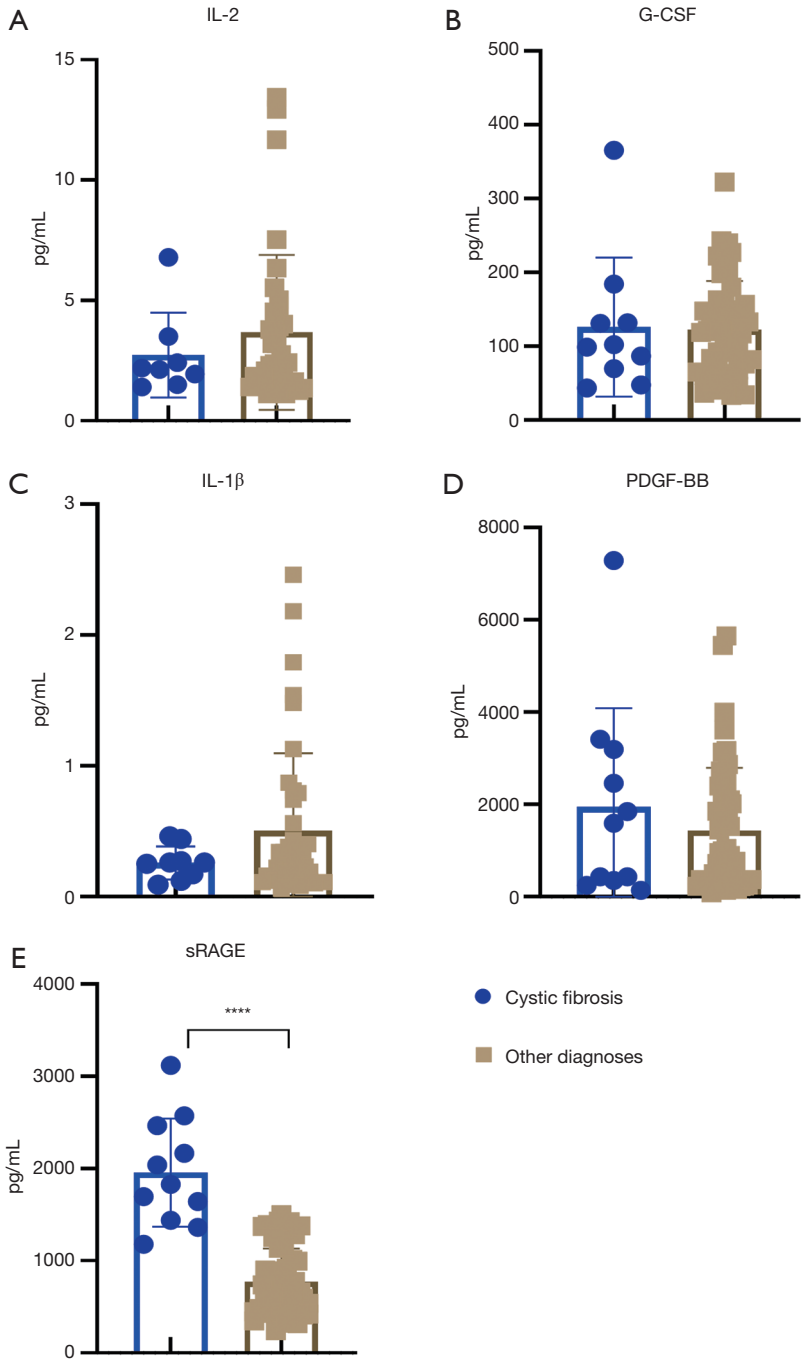

Figure 4 Bar plots of serum cytokines in patients diagnosed with cystic fibrosis versus all other baseline diagnosis. (A) IL-2, (B) G-CSF, (C) IL-1β, (D) PDGF-BB, and (E) sRAGE. ****, P<0.00001. IL, interleukin; G-CSF, granulocyte colony-stimulating factor; PDGF, platelet-derived growth factor.

clinicians gain a better understanding of the effects of the refinements in the updated guidelines in associating PGD with risk factors and clinical outcomes. As expected, we showed that a PGD diagnosis by either guideline resulted in significant morbidity and mortality. This is consistent with previous reports indicating the discriminative ability

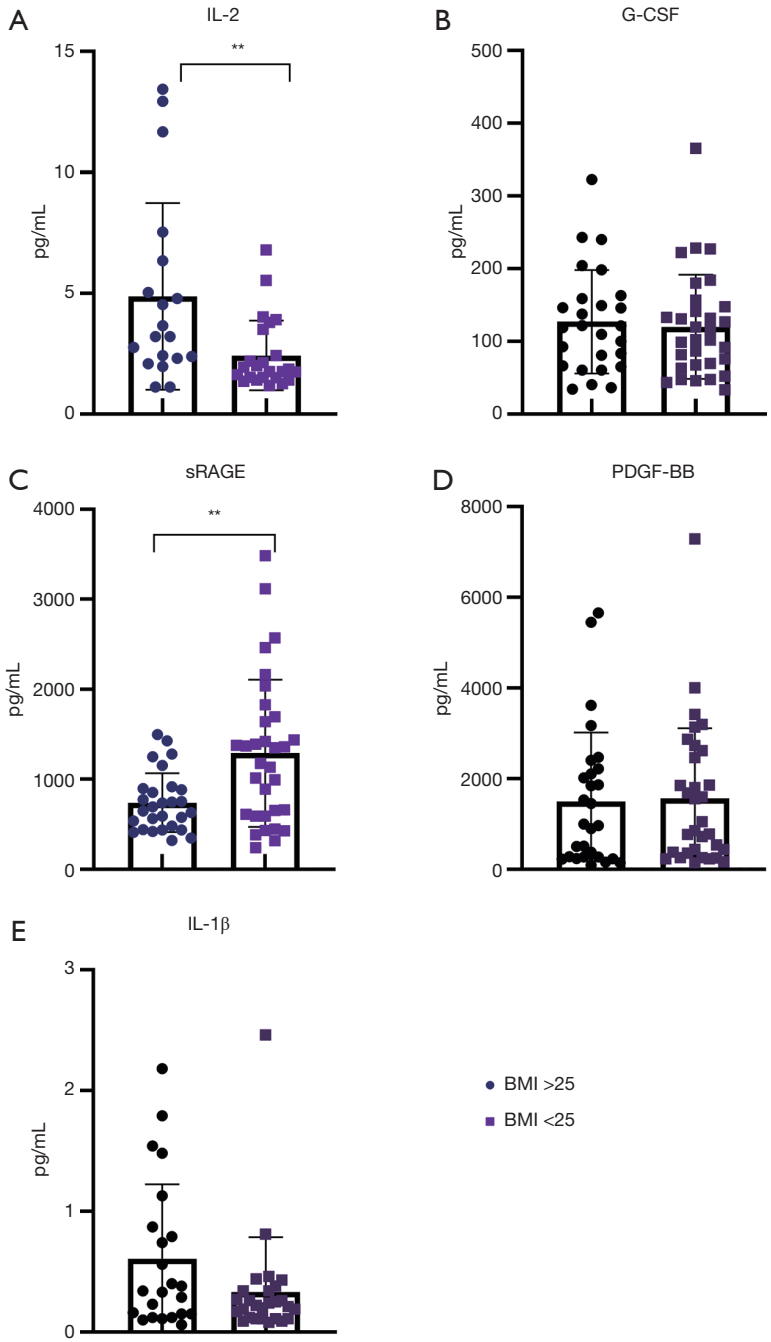

Figure 5 Bar plots of serum cytokines in patients with BMI >25 versus BMI <25. (A) IL-2, (B) G-CSF, (C) sRAGE, (D) PDGF-BB, and (E) IL-1β. **, P<0.001. BMI, body mass index; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; PDGF, platelet-derived growth factor.

of arterial oxygen partial pressure to fractional inspired oxygen ratios (PaO_2/FiO_2) at 48–72 hours post-transplant to show differences in mortality regardless of ventilation strategy (6,11). More cases of PGD were diagnosed by using the 2016 guidelines, mostly due to the clarified grading criteria for extubated patients that were associated with greater sensitivity in identifying risk factors for PGD such as ECLS use, recipient BMI, and diagnosis of restrictive lung disease. The limited numbers and decreased sensitivity of these factors in the 2005 guidelines resulted in

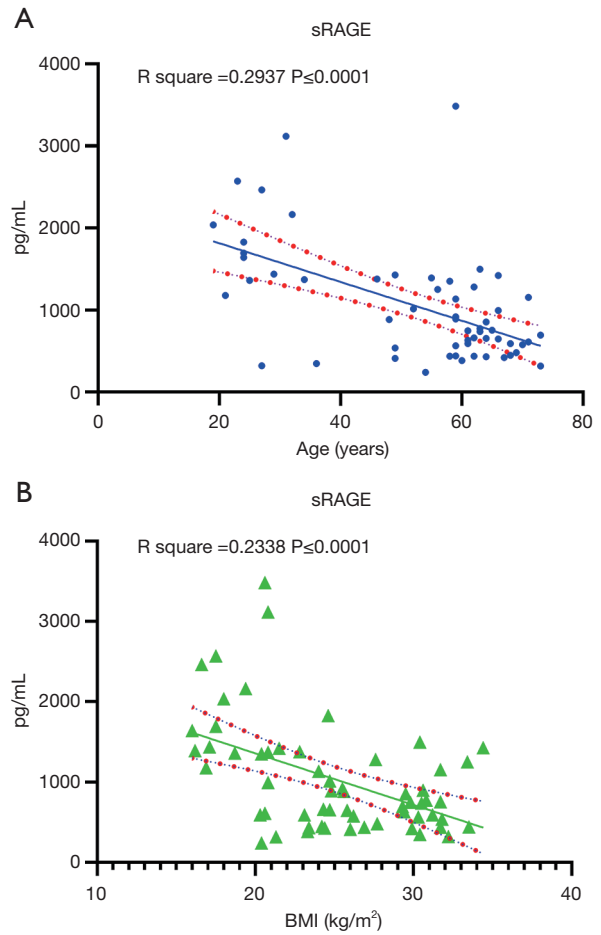

Figure 6 Linear regression graphs showing correlation between sRAGE and patient clinical characteristics. (A) Correlation between sRAGE levels and age, (B) correlation between sRAGE and BMI. BMI, body mass index.

a weaker association in our single-center cohort. Biological risk factors were also more prevalent in the 2016 cohort than in the 2005 cohort, although no biomarkers were independently associated with PGD after adjusting for clinical risk factors.

In 2013, the Lung Transplant Outcomes Group (LTOG), using the 2005 PGD scoring system (5), reported a 16.8% incidence of PGD3 at 48 or 72 hours, which was strongly associated with an increased 90-day and 1-year mortality (5). Using the 2005 PGD scoring system, we reported a 26% incidence of PGD3 in a smaller single center cohort. However, using the updated 2016 PGD guidelines with specific grading protocols for extubated patients, we found a 37% incidence of PGD3 at 48–72 hours. These rates were higher than those reported by the LTOG in 2013, but our 1-year survival rates were similar. Extended criteria donor was used in 35% of cases and ECLS in 89% of cases. Both factors, along with the updated guidelines, could have led to a higher PGD rate. Of note, our ECLS practice has evolved from use of cardiopulmonary bypass, a known risk factor for PGD, to the use of ECMO, considered by some to reduce PGD (5,12). In general, we use ECLS to improve intraoperative hemodynamics and exposure when needed.

Consistent with previous reports, we found that a designation of PGD according to the updated 2016 consensus was significantly associated with several adverse events including prolonged hospital and ICU length of stays. In addition, patients with PGD had a prolonged intubation period and required a tracheostomy more often than did those without PGD. Moreover, PGD patients were more likely to require dialysis and postoperative ECMO and to have a peak creatinine ≥1.5 mg/dL within 48 hours of reperfusion. Our findings support those of others suggesting considerable cross talk between the lungs and kidney (13). Impaired gas exchange and increased cytokines from lung injury can lead to renal microvascular dysregulation, apoptosis, and endothelial dysfunction. The lack of renal fluid and solute clearance can exacerbate pulmonary dysfunction and edema.

In our study, 90-day mortality for patients with PGD was only 8.5%, which is substantially better than previous reports (5,14). However, the likelihood of dying in the hospital was 6-fold higher in those with PGD than in those without it. In addition, the 1-year mortality was 25.5% for PGD patients and 5% for those without it. These findings were also seen with the 2005 guidelines, suggesting a similar impact of PGD by either scoring system (5). Importantly, our analysis did demonstrate that the subgroup of patients who were designated as PGD3 at 48–72 hours while extubated fared better than those who were intubated. However, this small subgroup analysis requires further study as it suggests that patients who are designated as PGD3 according to the 2016 guidelines may have a better prognosis if they are extubated at 48–72 hours. Whether this reflects a mechanistic link between lower barotrauma and better lung healing from the avoidance of mechanical ventilation or is simply a marker of less severe lung injury requires further investigation.

Previous reports have linked baseline biomarker levels in recipients with the risk of PGD using early PGD scoring definitions (15). In evaluating pre-transplant levels of circulating cytokines and chemokines, we noted a trend toward greater inflammation in patients who eventually

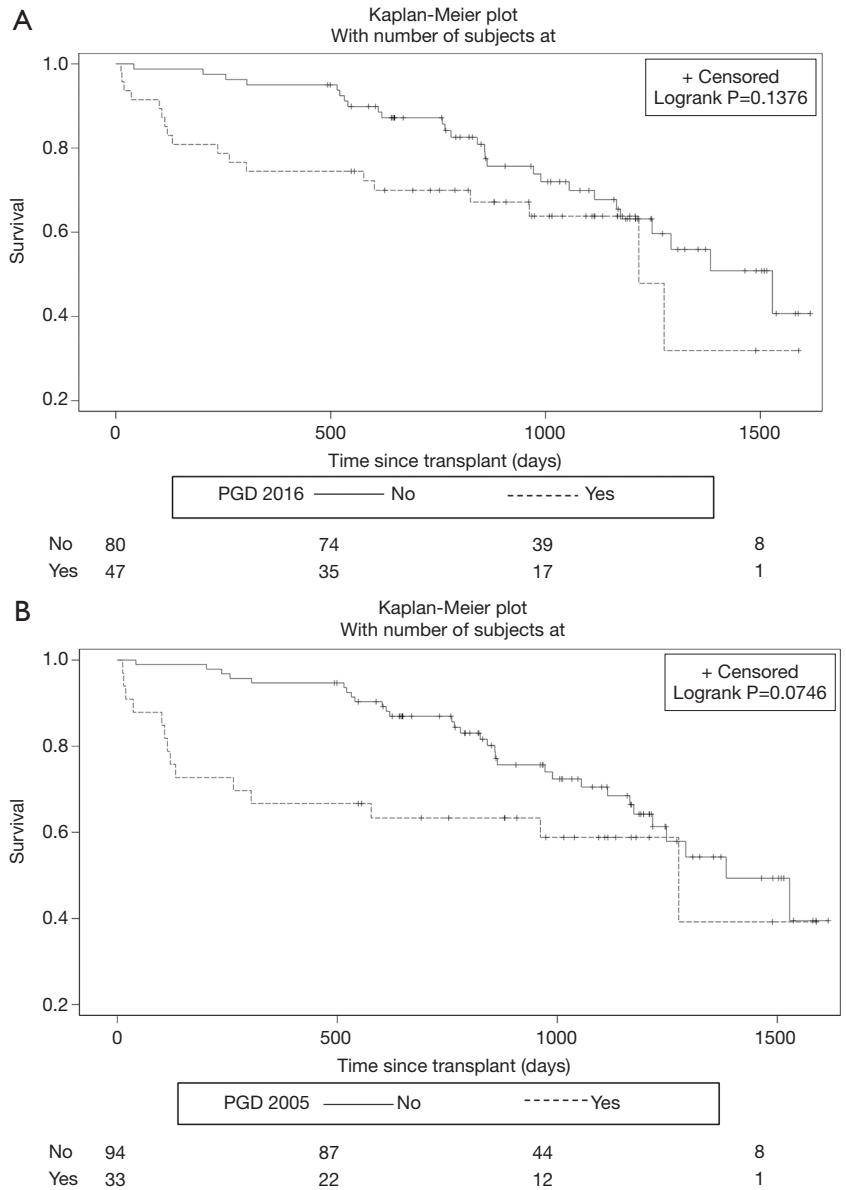

Figure 7 Kaplan-Meier plots of survival estimate for patients without and with PGD. (A) Survival estimate using the 2016 scoring guidelines, (B) survival estimate using the 2005 scoring guidelines. PGD, primary graft dysfunction.

developed PGD (*Figure 1F*). Three biomarkers were significantly associated with PGD on univariate analysis: pre-transplant IL-2 and PDGF-BB levels were higher and sRAGE levels were lower. ROC and multivariate analysis of cytokines suggested that IL-2 and sRAGE were the most predictive of PGD. Only IL-2 was predictive of PGD with the 2005 guidelines, suggesting the updated guidelines may be more sensitive at detecting preoperative molecular risk factors. However, no biomarker was independently associated with PGD development after adjusting for clinical risk factors.

PGD pathophysiology involves an ischemia-reperfusion–mediated injury to alveolar, endothelial, and interstitial cells, followed by the migration of polymorphonuclear neutrophils from the pulmonary circulation into the interstitium and airways (16,17). Immune responses to pulmonary injury ultimately exacerbate lung damage and lead to the development of clinical PGD. It is reasonable

to hypothesize that a recipient's baseline inflammatory state may predict PGD development. Although the underlying mechanism is unclear, both innate and adaptive immunity rely on cytokine signaling to enhance activity. A greater baseline milieu of cytokine signals could fuel immune-related events leading to PGD. For example, IL-2 enhances immune activity via multiple pathways (18), so patients with elevated IL-2 levels may be primed for a heightened inflammatory response that could exacerbate PGD injury.

Levels of sRAGE were lower in patients who developed PGD. RAGE is a transmembrane receptor of the immunoglobulin superfamily that is highly expressed in the lungs (19). RAGE activation modulates cell signaling that enhances inflammatory responses. Furthermore, Christie *et al.* showed that sRAGE levels were higher 6 and 24 hours after reperfusion in patients who developed PGD, although baseline measurements were not reported (20). Pelaez *et al.* reported that higher sRAGE concentrations in donor levels before transplant and recipient levels after transplantation were associated with PGD development (21), but again pretransplant levels were not measured in recipients. Others have shown that elevated sRAGE levels early during reperfusion were predictive of increased PGD and adverse events using the 2005 guidelines (20,22).

It is challenging to explain why patients at risk of PGD would have lower levels of sRAGE. One possible explanation is, as we and others have shown, that sRAGE was decreased in patients with idiopathic pulmonary fibrosis (which was associated with PGD in our study) as compared with controls (23-25). Interestingly, sRAGE levels may be higher in patients with COPD (26) and in cystic fibrosis (27); in our study, only 9% of patients with cystic fibrosis and less than 20% of COPD patients developed PGD. This could explain the higher levels of sRAGE in non-PGD patients.

Using the 2016 consensus guidelines, we found an association between several baseline biomarkers and known PGD risk factors. Patients with restrictive lung disease had significantly lower levels of sRAGE than did patients with other diseases. Patients with a BMI >25 kg/m^2 had significantly lower sRAGE levels and higher IL-2. These findings suggest that clinical risk factors for PGD such as increased BMI and underlying disease may contribute to the inflammation that primes patients for developing PGD under the right circumstances.

This retrospective single-center study has several limitations. To mitigate the biases of retrospective data collection, the data were cross-referenced to data collected for regulatory reasons and were deemed consistent and accurate. Also, PGD was scored by an expert single grader with access to all relevant respiratory, blood gas, and CXR data. However, the timing of the collection of arterial blood gas samples, CXRs, and ventilator parameters was not standardized. Our comparison of the two guidelines resulted in differences related to the grading of extubated patients. We did not have ECMO cases for non-hypoxic indications or ECMO with clear CXR, which would be considered un-gradable in the 2016 guidelines. Thus, we cannot make conclusions about the effects of the updated ECMO grading on the reported incidence and risk factors of PGD. Moreover, serum samples were stored at –80 ℃ and may have deteriorated over time. The number of samples was limited due to budget constraints and availability of those obtained within 3 days of transplant. We excluded the few patients who underwent multi-organ and single-lung transplants from the study cohort of 60 patients to yield a more homogeneous group. However, clinical characteristics of this group of 60 patients were representative of the larger cohort including more patients with restrictive lung disease and larger BMIs in the PGD group (Table S2). Also, clinical outcomes were representative of the larger cohort including greater adverse events in the PGD group (Table S4). Multiplex experiments were used because of their potential to yield a broader molecular phenotype using a low sample volume; however, they are subject to false positives due to the multiple comparisons used in the statistical analysis. Also, four biomarkers were below the detection range (GM-CSF, IL-5, vascular endothelial growth factor, IL-15), making it difficult to interpret their relationship with PGD.

The grading of PGD in single lung transplants is controversial. The native contralateral lung could protect the allograft from causing systemic hypoxia; however, it could also exacerbate hypoxia. Previous analysis identified single lung transplants as an important risk factor for PGD (5). The 2016 ISHLT consensus guidelines suggest reporting the rates individually, as done in *Table 1*, but they do not suggest changing the scoring of PGD in single lungs. For the pooled analysis of outcomes and risk factors for PGD, we did not exclude single lung transplants as they represent 12% of the overall cohort and could substantially bias our results. We did carefully review each single lung CXR and grade the PGD according to the systemic PaO_2 or saturation scale in accordance with the guidelines. Importantly, we did exclude single lung transplants from our analysis of the effect of baseline cytokine levels on the development of PGD. This was done to reduce heterogeneity in the smaller subgroup analysis

and to reduce the potential bias of residual inflammatory contribution from the remaining contralateral lung.

In conclusion the current analysis highlights improvements in the updated PGD scoring guidelines, which still predict substantial adverse effects of PGD on patient outcomes but also increase the detection of PGD and clinical and molecular risk factors. Thus, the new scoring system is more sensitive, which has important research implications and may improve the ability to detect risk factors, but the clinical implications of having a sensitive scoring system may not be as severe if the patient remains extubated at 48–72 hours post-transplant.

We recommend using the updated 2016 PGD scoring guidelines in all lung transplant recipients, independent of intubation status, as a vital performance and research metric. Reporting and analysis of these scores could lead to improved detection, prevention, and management of PGD. The inverse correlations between sRAGE levels and PGD and between sRAGE and clinical risk factors for PGD are novel findings that warrant further study. Despite the potential mechanistic insight gained from preoperative biomarkers and the potential for identifying molecular phenotypes of PGD, clinical risk factors alone appear to be sufficient for predicting patients at greater risk.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jtd-20-3564). GL reports grants from Transmedics, Inc., during the conduct of the study; grants from Abiomed, related travel/housing reimbursement from Transmedics, Inc., grants from Medtronic, grants from Abbott, outside the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics board at Baylor College of Medicine (No. 00000286) with waiver of consent due to the retrospective nature of the study. The data was anonymized for the privacy of the participants.

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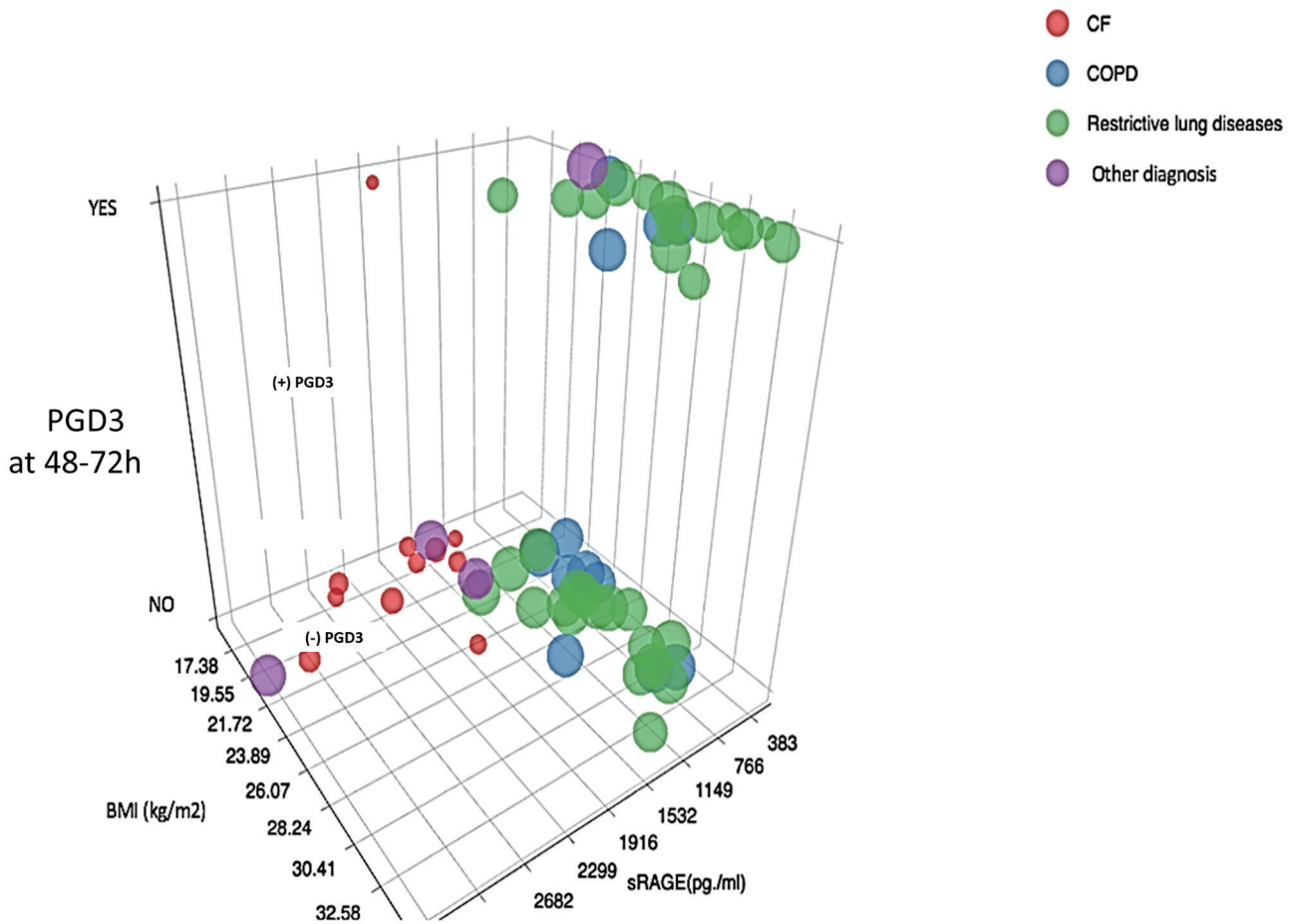

Figure S1 Relationship between underlying disease, body mass index (BMI), age (circle size), and presence or absence of PGD3 and sRAGE concentration. CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease.

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Table S1 Baseline cytokines using 2005 and 2016 guidelines (n=60; HLA samples)

Cytokines	2005 Guidelines			2016 Guidelines		
	Non-PGD (n=46)	PGD (n=14)	P	Non-PGD (n=39)	PGD (n=21)	P
MIP-1B	41.34±53.42	44.15±29.18	0.208	43.41±57.58	39.36±25.85	0.540
IL-6	7.91±18.17	2.31±3.87	0.703	7.59±17.77	4.62±9.25	0.591
IL-1RA	761.51±1,847.38	842.36±1,961.00	0.442	626.86±1,454.63	1,065.47±2,455.67	0.296
TNF-α	12.36±14.83	12.90±8.61	0.287	12.86±15.77	11.80±8.54	0.967
Rantes	6,035.28±8,018.89	7,970.43±9,654.13	0.207	5,736.44±8,027.56	7,794.98±9,060.21	0.158
IL-2	3.00±2.30	6.65±6.45	0.048	2.94±2.47	4.55±3.69	0.018
IL-1β	0.42±0.55	0.59±0.55	0.351	0.37±0.46	0.63±0.65	0.090
Eotaxin	77.19±93.28	77.66±83.15	0.766	63.16±77.53	103.57±107.42	0.196
Basic FGF	12.77±11.97	15.70±12.18	0.270	12.49±12.41	15.33±11.19	0.175
PDGF-BB	1,438.74±1,528.00	1,834.37±1,524.54	0.311	1,268.99±1,446.40	2,017.75±1,578.12	0.045
IP-10	366.63±440.70	473.87±465.75	0.421	378.85±471.79	415.43±400.43	0.403
IL-13	1.08±1.04	1.49±1.72	0.828	0.90±0.57	1.60±1.78	0.389
IL-4	1.56±1.31	1.51±1.18	0.877	1.38±1.17	1.90±1.40	0.139
MCP-1	38.96±75.08	26.63±17.58	0.354	38.34±80.34	31.45±23.14	0.097
IL-8	157.01±243.76	122.74±211.38	0.943	145.14±228.98	156.15±252.77	0.433
MIP-1A	2.36±3.73	3.04±2.39	0.120	2.47±4.01	2.59±2.12	0.181
G-CSF	118.82±73.74	138.54±59.74	0.174	109.84±66.38	148.24±73.55	0.028
IL-12p70	4.54±7.21	5.11±8.68	0.759	4.39±7.77	5.18±7.70	0.366
IL-17A	7.76±6.21	12.36±12.90	0.431	7.73±6.61	11.03±10.98	0.204
IL-9	28.91±11.41	36.94±38.65	0.637	28.93±9.81	34.23±33.11	0.278
sRAGE	1,102.88±721.34	807.87±544.27	0.108	1,170.43±753.84	780.77±476.07	0.024

Values are mean ± standard deviation. FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; IP, interferon-inducible protein; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; sRAGE, soluble form of the receptor for advanced glycation end-products; TNF, tumor necrosis factor.

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Table S2 Demographics and risk factors for patients who had preoperative HLA samples tested for cytokine analysis using 2005 and 2016 guidelines

Variable	Total (n=60)	2005 Guidelines			2016 Guidelines		
		Non-PGD (n=46)	PGD (n=14)	P	Non-PGD (n=39)	PGD (n=21)	P
Recipient characteristics							
Female	30 (50.0)	25 (54.35)	5 (35.71)	0.360	21 (53.85)	9 (42.86)	0.589
Age (years)	53.08±15.87	52.65±15.93	54.5±16.18	0.706	52.1±16.7	54.4±14.56	0.519
BMI (kg/m^2)	25.06±5.21	24.21±5.08	27.85±4.75	0.021	23.46±4.95	28.03±4.38	0.0008
Blood type			0.280				0.222
A	24 (40.0)	16 (34.78)	8 (57.14)		14 (35.90)	10 (47.62)	
B	5 (8.33)	5 (10.87)	0 (0.0)		5 (12.82)	0 (0.0)	
AB	3 (5.0)	3 (6.52)	0 (0.0)		3 (7.70)	0 (0.0)	
O	28 (46.67)	22 (47.83)	6 (42.86)		17 (43.59)	11 (52.38)	
Rh group negative	8 (13.33)	5 (10.87)	3 (21.43)	0.374	4 (10.26)	4 (19.05)	0.433
Primary disease				0.418			0.138
Restrictive lung disease	32 (53.33)	22 (47.83)	10 (71.43)		17 (43.59)	15 (71.43)	
Cystic fibrosis	11 (18.33)	10 (21.74)	1 (7.14)		10 (25. 64)	1 (4.76)	
COPD	13 (21.67)	11 (23.91)	2 (14.29)		9 (23.08)	4 (19.05)	
Other	4 (6.67)	3 (6.52)	1 (7.14)		3 (7.70)	1 (4.76)	
Primary disease				0.140			0.058
Restrictive lung disease	32 (53.33)	22 (47.83)	10 (71.43)		17 (43.59)	15 (71.43)	
Non-restrictive lung diseases	28 (46.67)	24 (52.17)	4 (28.57)		22 (56.41)	6 (28.57)	
Diabetes	10 (16.67)	8 (17.39)	2 (14.29)	1.000	7 (17.95)	3 (14.29)	1.000
Hypertension	26 (43.33)	20 (43.48)	6 (42.86)	1.000	16 (41.03)	10 (47.62)	0.785
History of smoking	28 (46.67)	22 (47.83)	6 (42.86)	0.770	18 (46.15)	10 (47.62)	1.00
LAS	42.24±12.26	41.34±10.44	45.22±17.10	0.304	41.14±10.14	44.29±15.53	0.347
Baseline creatinine (mg/dL)	0.83±0.19	0.81±0.18	0.87±0.23	0.323	0.81±0.18	0.85±0.21	0.427
Condition at time of transplant				0.905			0.759
Not hospitalized	52 (86.67)	40 (86.96)	12 (85.71)		33 (84.62)	19 (90.48)	
Hospitalized (not in ICU)	5 (8.33)	4 (8.70)	1 (7.14)		4 (10.26)	1 (4.76)	
In ICU	3 (5.0)	2 (4.35)	1 (7.14)		2 (5.13)	1 (4.76)	
Life support before transplant	4 (6.67)	2 (4.35)	2 (14.29)	0.230	2 (5.13)	2 (9.52)	0.606
Pre-operative ECMO	2 (3.33)	1 (2.17)	1 (7.14)	0.415	1 (2.56)	1 (4.76)	1.00
Pulmonary hypertension	49 (81.67)	38 (82.61)	11 (78.57)	0.708	32 (82.05)	17 (80.95)	1.00
Prior thoracic surgery*	10 (16.67)	7 (15.22)	3 (21.43)	0.685	4 (10.26)	6 (28.57)	0.143
Prior cardiac surgery	1 (1.67)	1 (2.17)	0 (0.0)	1.000	1 (2.56)	0 (0.0)	1.00
Prior pleurodesis	6 (10.0)	3 (6.52)	3 (21.43)	0.133	3 (7.70)	3 (14.29)	0.655
Donor characteristics							
Age (years)	33.6±12.73	33.87±12.73	32.71±13.89	0.769	33.15±12.87	34.43±12.71	0.714
Extended criteria donor†	19 (31.67)	12 (26.09)	7 (50.00)	0.092	11 (28.21)	8 (38.10)	0.432
Smoker ever	31 (51.67)	24 (52.17)	7 (50.0)	1.000	21 (53.85)	10 (47.62)	0.788
Smoker >20PYH	5 (8.33)	4 (8.70)	1 (7.14)	1.000	4 (10.26)	1 (4.76)	0.649
DCD	5 (8.33)	3 (6.52)	2 (14.29)	0.582	2 (5.13)	3 (14.29)	0.332
Operative characteristics							
Bilateral type of transplant	60 (100.0)	46 (100.0)	14 (100.0)		39 (100.0)	21 (100.0)	
Type of intraoperative support				1.000			1.000
Off-pump	1 (1.67)	1 (2.17)	0 (0.0)		1 (2.56)	0 (0.0)	
ECLS	59 (98.33)	45 (97.83)	14 (100.0)		38 (97.44)	21 (100.0)	
Type of ECLS				1.000			1.000
ECMO	4 (6.67)	3(6.67)	1(7.14)		3 (7.89)	1 (4.76)	
CPB	55 (93.22)	42 (93.33)	13 (92.86)		35 (92.11)	20 (95.24)	
Total ischemic time (min)	295.77±63.59	290.15±66.33	314.2±51.41	0.218	296.15±65.34	295.05±61.77	0.949

Values are n (%) or mean ± SD. *Prior thoracic surgery (non-transplant). †One or more of the following: age >55 years, anticipated ischemia >6 hours, DCD, PaO2/FiO2 <300, donor is >20PYH smoker. COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; PGD, primary graft dysfunction; PYH, pack-year history; CPB, cardiopulmonary bypass; DCD, donor after cardiac death; LAS, lung allocation score.

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Table S3 Clinical characteristics, outcomes, and biomarkers in patients with PGD according to 2005 and 2016 guidelines and in those downgraded to PGD1 using the 2005 guidelines

Variable	PGD 2016 & 2005 (n=33)	PGD 2016 not 2005 (n=14)	P
Recipient characteristics			
Women	10 (30.30)	6 (42.86)	0.406
Age	54.45±15.85	55.50±12.81	0.828
BMI (kg/m^2)	26.15±5.15	27.41±4.13	0.423
Primary disease			0.243
Restrictive lung disease	24 (72.72)	7 (50.50)	
Non-restrictive lung diseases	9 (27.28)	7 (50.50)	
Diabetes	11 (33.33)	4 (28.57)	1.00
Hypertension	17 (51.52)	9 (64.29)	0.421
History of smoking	16 (48.48)	9 (64.29)	0.321
Lung allocation score	45.35±14.17	41.03±9.43	0.302
Baseline creatinine (mg/dL)	0.85±0.20	0.89±0.21	0.639
Condition at time of transplant			0.582
Not hospitalized	28 (84.85)	14 (100.0)	
Hospitalized (not in ICU)	2 (6.06)	0 (0)	
In ICU	3 (9.09)	0 (0)	
Life support before LTx	4 (12.12)	0 (0)	0.302
Pre-operative ECMO	1 (3.03)	0 (0)	0.7
Pulmonary hypertension	23 (69.70)	13 (92.86)	0.181
Prior thoracic surgery (non-transplant)	6 (18.18)	3 (21.43)	1.00
Prior cardiac surgery	0 (0)	1 (7.14)	0.298
Prior pleurodesis	3 (9.09)	0 (0)	0.544
Prior lung transplant	1 (3.03)	1 (7.14)	0.879
Donor characteristics			
Age (years)	33.67±12.88	38.21±10.39	0.249
Extended criteria donor	16 (48.48)	4 (28.57)	0.334
Smoker ever	19 (57.58)	7 (50.00)	0.633
Sex mismatch	12 (36.36)	5 (35.71)	0.772
Sex mismatch, female-male	9 (27.28)	2 (14.29)	0.559
Donor after cardiac death	6 (18.18)	1 (7.14)	0.657
Ex-vivo lung perfusion	3 (9.09)	1 (7.14)	1.00
Operative characteristics			
Type of transplant			0.627
Bilateral	30 (90.91)	12 (85.71)	
Single	3 (9.09)	2 (14.29)	
Type of intraoperative support			0.298
Off-pump	0 (0)	1 (7.14)	
ECLS	33 (100.0)	13 (92.86)	
Type of ECLS			0.849
Cardiopulmonary bypass	28 (84.85)	12 (92.31)	
Modified bypass	5 (15.15)	1 (7.69)	
Total ischemic time (min)	346.30±118.45	301.5±128.44	0.253
Outcome			
Postoperative length of stay (days)	46.30±40.97	20.93±13.47	0.029
ICU length of stay (days)	37.33±36.07	11.71±13.33	0.135
Peak creatinine within 48 hours (mg/dL)	1.23±0.59	0.93±0.19	0.073
Peak creatinine value <1.5 mg/dL	25 (75.76)	14 (100.0)	0.084
Peak creatinine value ≥1.5 mg/dL	8 (24.24)	0 (0)	0.084
Max creatinine (mg/dL)	1.96±1.40	1.48±0.85	0.260
Max creatinine <1.5 mg/dL	15 (45.45)	11 (78.57)	0.055
Max creatinine ≥1.5 mg/dL	18 (54.55)	3 (21.43)	0.055
Discharge creatinine (mg/dL)	1.05±1.05	0.98±0.89	0.420
Peak lactate within 72 hours (mg/dL) (n=115)	9.02±3.64	5.49±3.16	0.004
Postoperative ECMO	15 (45.45)	0 (0)	0.002
Mechanical ventilation ≥48 hours	29 (87.88)	3 (21.43)	0.000
Mechanical ventilation ≥5 days	22 (66.67)	1 (7.14)	0.000
Reintubated	11 (33.33)	2 (14.29)	0.288
Tracheostomy	18 (54.55)	1 (7.14)	0.003
Airway dehiscence	3 (9.09)	0 (0)	0.544
Dialysis	12 (36.36)	0 (0)	0.009
Discharge disposition			0.164
Home	18 (54.55)	11 (78.57)	
Rehabilitation facility	8 (24.24)	3 (21.43)	
Death	7 (21.21)	0 (0)	
90-day mortality	4 (12.12)	0 (0)	0.302
1-year mortality	11 (33.33)	1 (7.14)	0.077
Biomarkers			
IL-2 (n=11 *vs.* 5)	6.65±6.45 pg/mL	3.30±1.06 pg/mL	0.276
IL-1β (n=12 *vs.* 6)	0.59±0.55 pg/mL	0.69±0.88 pg/mL	0.771
PDGF-BB (n=14 *vs.* 7)	1834.37±1524.54 pg/mL	2384.50±1740.78 pg/mL	0.457
G-CSF (n=14 *vs.* 7)	211.20±377.85 pg/mL	166.25±97.02 pg/mL	0.685
sRAGE (n=14 *vs.* 7)	807.87±544.27 pg/mL	726.56±329.39 pg/mL	0.722

Values are mean ± standard deviation. PGD 2016 & 2005, patients who developed PGD3 at 48-72 hours after transplant using the 2016 and 2005 scoring guidelines; PGD 2016 not 2005, patients who developed PGD3 at 48-72 hours after lung transplant using the 2016 guidelines but not when using the 2005 guidelines. IL, interleukin; PDGF, platelet-derived growth factor; GCSF, granulocyte colony stimulating forming; sRAGE, soluble form of the receptor for advanced glycation end-products.

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Table S4 Outcomes for 60 patients who had preoperative HLA samples tested for cytokine analysis using 2005 and 2016 guidelines

Variable	Total (n=60)	2005 Guidelines			2016 Guidelines		
		Non-PGD (n=46)	PGD (n=14)	P	Non-PGD (n=39)	PGD (n=21)	P
Postoperative length of stay (days)				0.047			0.178
≤21	31 (51.67)	26 (56.52)	5 (35.71)		21 (53.85)	10 (47.62)	
22-30	14 (23.33)	12 (26.09)	2 (14.29)		11 (28.21)	3 (14.29)	
>30	15 (25.00)	8 (17.39)	7 (50.0)		7 (17.95)	8 (38.10)	
ICU length of stay (days)	18.85±27.24	15.06±27.24	31.29±35.27	0.050	15.1±24.55	25.81±31.07	0.148
Peak creatinine within 48 hours (mg/dL)	1.05±0.48	1.00±0.48	1.22±0.45	0.140	1.02±0.51	1.11±0.41	0.526
Peak creatinine within 48 hours ≥1.5 (mg/dL)	6 (10.0)	2 (4.35)	4 (28.57)	0.023	2 (5.13)	4 (19.05)	0.171
Max creatinine (mg/dL)	1.46±1.16	1.43±1.16	1.56±0.83	0.710	1.47±1.31	1.47±0.84	0.954
Max creatinine ≥1.5 (mg/dL)	17 (28.33)	10 (21.74)	7 (50.0)	0.040	9 (23.08)	8 (38.10)	0.176
Discharge creatinine (mg/dL)	0.87±0.51	0.87±0.51	0.86±0.37	0.930	0.91±0.59	0.84±0.39	0.479
Peak lactate within 72h (mg/dL) (n=55)	7.12±3.51	6.54±3.51	8.81±3.04	0.036	6.62±3.59	7.99±3.27	0.164
Postoperative ECMO	4 (6.64)	1 (2.17)	3 (21.43)	0.036	1 (2.56)	3 (14.29)	0.119
Mechanical ventilation ≥48 h	28 (46.67)	16 (34.78)	12 (85.71)	0.002	14 (35.90)	14 (66.67)	0.031
Mechanical ventilation ≥5 days	18 (30.0)	9 (19.57)	9 (64.29)	0.003	8 (20.51)	10 (47.62)	0.040
Reintubated	19 (31.67)	15 (32.61)	4 (28.57)	1.000	14 (35.90)	5 (23.81)	0.254
Tracheostomy	15 (25.0)	9 (19.57)	6 (42.86)	0.092	8 (20.51)	7 (33.33)	0.352
Airway dehiscence	1 (1.67)	0 (0.0)	1 (7.14)	0.233	0 (0.0)	1 (4.76)	0.350
Dialysis	6 (10.0)	4 (8.70)	2 (14.29)	0.617	4 (10.26)	2 (9.52)	1.00
Discharge disposition				0.312			0.1743
Home	46 (76.67)	37 (80.43)	9 (64.29)		33 (84.62)	13 (61.90)	
Rehabilitation facility	13 (21.67)	8 (17.39)	5 (35.71)		6 (15.38)	7 (33.33)	
Death	1 (1.67)	1 (2.17)	0 (0.0)		1 (2.56)	0 (0.0)	
Readmission within 1 year (n=59)	50 (84.75)	39 (86.67)	11 (78.57)	0.431	33 (86.84) n=38	17 (80.95)	0.708
ICU readmission within 1 year (n=59)	20 (33.9)	14 (31.11)	6 (42.86)	0.521	11 (28.95) n=38	9 (42.86)	0.390
90-day mortality	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
1-year mortality	4 (6.64)	3 (6.52)	1 (7.14)	1.000	2 (5.13)	2 (9.52)	0.606

Values are n (%) or mean ± SD. ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

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