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Brief Communication

Preoperative Plasma Club (Clara) Cell Secretory Protein Levels Are Associated With Primary Graft Dysfunction After Lung Transplantation

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Inherent recipient factors, including pretransplant diagnosis, obesity and elevated pulmonary pressures, are established primary graft dysfunction (PGD) risks. We evaluated the relationship between preoperative lung injury biomarkers and PGD to gain further mechanistic insight in recipients. We performed a prospective cohort study of recipients in the Lung Transplant Outcomes Group enrolled between 2002 and 2010. Our primary outcome was Grade 3 PGD on Day 2 or 3. We measured preoperative plasma levels of five biomarkers (CC-16, sRAGE, ICAM-1, IL-8 and Protein C) that were previously associated with PGD when measured at the postoperative time point. We used multivariable logistic regression to adjust for potential confounders. Of 714 subjects, 130 (18%) developed PGD. Median CC-16 levels were elevated in subjects with PGD (10.1 vs. 6.0, $p < 0.001$). CC-16 was associated with PGD in nonidiopathic pulmonary fibrosis (non-IPF) subjects (OR for highest quartile of CC-16: 2.87, 95% CI: 1.37, 6.00, $p = 0.005$) but not in subjects with IPF (OR 1.38, 95% CI: 0.43, 4.45, $p = 0.59$). After adjustment, preoperative CC-16 levels remained associated with PGD (OR: 3.03, 95% CI: 1.26, 7.30, $p = 0.013$) in non-IPF subjects. Our study suggests the importance of preexisting airway epithelial injury in PGD. Markers of airway epithelial injury may be helpful in pretransplant risk stratification in specific recipients.

Keywords: Acute lung injury, biomarkers, CC-16, lung transplantation, primary graft dysfunction

Abbreviations: ALI, acute lung injury; AUC, area under the curve; CC-16, club (Clara) cell secretory protein; COPD, chronic obstructive pulmonary disease; FiO_2, fraction of inspired oxygen; ICAM-1, intercellular adhesion molecule-1; IPF, idiopathic pulmonary fibrosis; mPAP, mean pulmonary arterial pressure; PGD, primary graft dysfunction; sRAGE, soluble receptor for advanced glycation end product; UNOS, United Network for Organ Sharing

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Introduction

Primary graft dysfunction (PGD) is a form of acute lung injury (ALI) occurring within 72 h of lung transplantation (1,2). It is the leading cause of early morbidity and mortality after transplant (3,4), yet the mechanisms driving the development of PGD remain unclear. Prior work evaluating postoperative time points has identified plasma biomarkers associated with concurrent PGD, including soluble receptor for advanced glycation end products (sRAGE), club (Clara) cell secretory protein (CC-16), Protein C and intercellular adhesion molecule-1 (ICAM-1) (5–8). These markers have helped establish potential mechanisms occurring during clinical PGD, and have demonstrated discriminant validity as a quantitative measure of PGD (9). However, there is a lack of knowledge of mechanisms occurring prior to transplant in the recipient that may be important in the development of PGD, and better preoperative recipient risk stratification may allow for changes in management or therapy prior to transplantation to reduce the risk of PGD.

Recently, we and others have established several recipient-related clinical risk factors for PGD, including obesity, presence of pulmonary hypertension and predisposing diagnosis (4). Identification of the biological processes underlying these clinical PGD associations is important because it will give insight into potentially modifiable factors prior to transplantation. For example, although predisposing diagnosis is not modifiable prior to transplantation, enhanced understanding of what is driving the increased risk of a particular diagnosis may provide targets for therapy to decrease PGD risk prior to transplantation. Additionally, studying biological markers within known risk groups is important as there are likely several different mechanisms contributing to the risk of PGD and measurement of biomarkers may allow for individualized management decisions to decrease PGD risk.

In order to further study potential mechanisms underlying previously established clinical risk factors and identify potential biological targets prior to transplantation to reduce the risk of development of PGD, we tested the association between five known PGD lung injury biomarkers measured preoperatively in the recipient and the subsequent risk of development of PGD.

Methods

Study population

The Lung Transplant Outcomes Group cohort is a multi-center, prospective study that has been previously described (5,6). In prior studies, we have measured postoperative biomarkers in smaller subsets of this cohort study (6–8). In this study, we measured five preoperative biomarkers in a large cohort of subjects that is expanded and distinct from previously studied cohorts. We included subjects transplanted between July 2002 and May 2010 with at least one biomarker measurement at the preoperative time point. The majority of samples were collected immediately prior to transplantation during the transplant admission; however, a fraction was collected at the time of listing. Samples were processed within 60 min and then stored at −80°C for subsequent analysis, and clinical data were collected prospectively for all subjects as described previously (5,7,10). Mortality information was collected from each center and supplemented with data from United Network for Organ Sharing (11). Institutional Review Board approval was obtained from each participating center. Informed consent was obtained from each subject enrolled in the cohort.

Determination of PGD grade

Our primary outcome was Grade 3 PGD at 48 or 72 h after transplantation. PGD grade was determined using the International Society for Heart and Lung Transplantation consensus definition (2,10,12). Two blinded physicians examined chest radiographs to assess for the presence of PGD. Radiographs qualified for PGD if the transplanted lung(s) had diffuse infiltrates. Radiographs and arterial blood gases were assessed at the time of admission to the ICU after transplantation (T0), and 24, 48 and 72 h after transplantation. The severity of PGD was graded according to the PaO_2/FiO_2 ratio, with a PaO_2/FiO_2 ratio less than 200 defining Grade 3 PGD (13).

Measurement of sRAGE, ICAM-1, Protein C, IL-8 and CC-16

Biomarkers were chosen because of previously reported associations with ALI or PGD (5,7,14,15). Protein C was measured using the Actichrome Protein C assay (American Diagnostica, Greenwich, CT). The intra-assay coefficient of variation was 5.5%. sRAGE, ICAM-1 and IL-8 were measured by ELISA (R&D, Minneapolis, MN). The intra-assay coefficients of variation were 7%, 5% and 3%, respectively. CC-16 levels were measured using a commercially available ELISA (Biovender, Candler, NC). The intra-assay coefficient of variation was 4%. All analytes were measured in duplicate.

Statistical analysis

Biomarkers were analyzed either continuously or using quartiles, based on fractional polynomial fit plots evaluating the relationship between each biomarker and predicted probabilities of PGD (16), as well as categorizing each biomarker into quartiles as a dummy variable in logistic regression models with PGD as the outcome. We evaluated CC-16 stratified by diagnosis (idiopathic pulmonary fibrosis [IPF] vs. non-IPF) based on our previous finding that diagnosis is an effect modifier of the relationship between CC-16 and PGD (8,17). We performed a sensitivity analysis of the association of biomarkers by time of collection, repeating the analyses in those subjects who had plasma collected at the time of listing, defined as greater than 24 h prior to transplantation.

We used multivariable logistic regression to evaluate the relationship between each biomarker and PGD while evaluating for confounding using variables previously demonstrated to be risk factors for PGD, including BMI, mean pulmonary arterial pressure (mPAP), transplant type, ischemic time, FiO_2 at reperfusion, female sex and parity, pretransplant diagnosis, donor smoking (defined as any history of smoking) and use of cardiopulmonary bypass (18). Using a prediction model previously developed (19) for PGD using bootstrap resampling methods, which incorporated pretransplant diagnosis, obesity and pulmonary artery pressure, we evaluated significant biomarkers for incremental predictive utility by comparing area under the curve (AUC) for the model with an individual biomarker to the model without. A likelihood ratio test was used to evaluate for significant differences in AUC. Multiple imputation was used to account for missing data in the covariates (20). Imputation was not used in either the exposure (biomarker) or the outcome (grade of PGD) variables; the very few individuals with missing biomarker values were excluded from analyses. p-Values of less than 0.05 were considered significant. Analyses were performed using STATA version 12.0 (STATA Corp., College Station, TX).

Results

There were 714 subjects in the study, of which 130 (18%, 95% CI: 15%, 21%) developed PGD. The majority of plasma samples were obtained at the time of transplantation; however, there were 126 subjects (19%) who had samples collected more than 24 h prior to transplantation. In those subjects, plasma samples were collected at the time of listing. The average time between collection and transplantation in those subjects was 80 ± 96 days. Missing biomarker values ($n = 2$) were due to assay failure.

Subjects with PGD more frequently received a lung from a smoking donor, were more obese and had IPF and PAH more often as a pretransplant diagnosis (10). Additionally, subjects with PGD had higher mPAP, more frequent red blood cell transfusions and more frequent use of cardiopulmonary bypass (Table 1). The percentage of missing data for each covariate is listed in Table 1. There were no significant differences in plasma levels of sRAGE, ICAM-1, IL-8 and Protein C between those with PGD and those without (Table 2).

Median plasma CC-16 levels were higher in subjects with PGD compared to those without (10.1 [IQR: 5.2, 19] vs. 6.0 [IQR: 3.4, 12.8], $p < 0.001$). We analyzed CC-16 categorically, in quartiles, based on the relationship of CC-16 with predicted probability of PGD generated from the fractional polynomial fit plot (Figure 1). There was an increased odds of PGD in subjects in the third quartile of CC-16 (OR: 1.89, 95% CI: 1.08, 3.32, $p = 0.03$) and the highest quartile of CC-16 (OR: 2.35, 95% CI: 1.35, 4.08, $p = 0.002$) compared to the lowest quartile of CC-16. When we evaluated CC-16 and pretransplant diagnosis (stratified as IPF vs. non-IPF), we found that the highest quartile of CC-16 had more subjects with IPF compared to other diagnoses (118 vs. 60, $p < 0.001$). There was no detectable association between CC-16 and PGD in subjects with IPF (OR for highest quartile of CC-16 1.38, 95% CI: 0.43, 4.45, $p = 0.59$). Plasma CC-16 levels were higher in subjects with PGD than without in the sub-group of non-IPF subjects (Figure 2A and B). The association between CC-16 and PGD was unchanged in subjects without IPF (OR for third quartile: 1.90 95% CI: 0.97, 3.72, $p = 0.06$ and highest quartile OR 2.87, 95% CI: 1.37, 6.01, $p = 0.005$). In a multivariable model with previously identified risk factors for PGD, the association between CC-16 and PGD in non-IPF subjects remained (OR for third quartile: 2.16, 95% CI: 1.00, 4.63, $p = 0.049$ and for fourth quartile: 3.03, 95% CI: 1.26, 7.30, $p = 0.013$; Table 3).

Given the association between preoperative plasma CC-16 and PGD, we evaluated CC-16 as a possible predictor for PGD in non-IPF subjects. First, we evaluated the predictive utility of CC-16 alone, which had an AUC of 0.60. Then, based on a previous study (19), we analyzed the predictive utility of pretransplant diagnosis, BMI category and mPAP as a base model. The negative predictive value of this model was 93%, and the positive predictive value was 20%. With the addition of CC-16 to the model, there were no significant improvements in the negative or positive predictive values (90% and 15%, respectively), despite statistically significant improvement in the AUC (0.72 for model with CC-16 vs. 0.70 for base clinical model, $p = 0.04$). Therefore, although elevated preoperative plasma CC-16 is an independent risk factor and possible biomarker of PGD, it may not be clinically useful in prediction of PGD when added to known clinical predictor variables.

In sensitivity analyses, the relationship between the biomarkers and the PGD did not change significantly by time of sample collection. In subjects who had samples collected at the time of transplantation, the relationship between CC-16 and PGD was unchanged (OR for highest quartile of CC-16: 2.07, 95% CI: 1.00, 4.31, $p = 0.05$). In subjects who had samples collected greater than 24 h from the time of transplantation ($n = 174$), there was no change in the relationship between CC-16 and PGD (OR for the highest quartile of CC-16 was 3.8, 95% CI: 1.19, 12.14, $p = 0.02$). There were not enough subjects to perform a stratified analysis by individual diagnosis category.

Discussion

In this study, we have demonstrated an association between plasma CC-16 levels measured preoperatively and PGD. This association was the strongest in subjects without IPF as a pretransplant diagnosis, and in subjects in the highest quartile of plasma CC-16. The association was independent of adjustment from multiple known confounding variables, indicating that the level of epithelial injury, as represented by circulating CC-16 levels, may predispose to PGD prior to the transplant procedure. Although CC-16 was not a good predictor of PGD, we have demonstrated the utility of CC-16 as a preoperative marker of PGD. This study builds on our prior work evaluating biomarkers in PGD (5,6,8) by exclusively evaluating the preoperative time point in a large cohort of prospectively studied transplant recipients, with adequate power to evaluate the role of biomarkers in prespecified sub-groups.

CC-16 is secreted by epithelial cells in the distal respiratory tract and acts to protect the integrity of the epithelial lining against inflammation and oxidant stress (21). In sarcoidosis, CC-16 is a biomarker of parenchymal disease severity, with increased levels being reflective of increasing parenchymal disease (15,22). CC-16 has also been evaluated as a biomarker of ALI, and plasma levels measured at the time of injury are decreased compared to other causes of pulmonary edema (23,24). In our study, increased plasma levels of CC-16 in the recipient prior to transplantation are associated with subsequent PGD. The difference in directionality from ALI may be because our measurement was taken prior to the development of lung injury, not during, indicating that preexisting epithelial injury is associated with subsequent graft dysfunction. It is possible that systemic up-regulation

Table 1: Univariate analysis of donor, recipient in perioperative variables stratified by primary graft dysfunction (PGD) status

Covariate	Number imputed, n (%)	PGD (n = 130)	Non-PGD (n = 584)	p-Value
Donor variables				
Male gender, n (%)	2 (0.3)	71 (55)	341 (58)	0.43
Age	7 (1)	35.4 ± 14.8	34.8 ± 14.1	0.70
Mode of death, n (%)	1 (<1)			0.87
Trauma		49 (38)	238 (41)	
Stroke		56 (43)	235 (40)	
Anoxia		9 (7)	46 (8)	
Other		16 (12)	65 (11)	
Race, n (%)	9 (1)			0.66
Caucasian		87 (67)	365 (63)	
African American		26 (20)	117 (20)	
Other		17 (13)	102 (17)	
Any smoking, yes	27 (4)	70 (54)	246 (43)	0.001
Recipient variables				
Male gender, n (%)	3 (<1)	73 (56)	311 (53)	0.55
Age	6 (1)	52.1 ± 12.7	53.0 ± 12.8	0.47
BMI	14 (2)	26.0 ± 4.7	24.6 ± 4.5	0.002
BMI category, n (%)				0.008
<18.5		11 (8)	59 (10)	
18.5–25		40 (31)	258 (44)	
25–30		52 (40)	196 (34)	
>30		27 (21)	71 (12)	
Pulmonary diagnosis, n (%)	3 (<1)			<0.001
Chronic obstructive pulmonary disease		33 (26)	256 (44)	
Idiopathic pulmonary fibrosis		56 (43)	180 (31)	
Cystic fibrosis		13 (10)	96 (16)	
Sarcoidosis (3)		3 (2)	15 (3)	
Pulmonary arterial hypertension (4)		13 (10)	15 (3)	
Other		12 (9)	22 (4)	
mPAP	136 (19)	35.0 ± 17.6	27.9 ± 10.2	<0.001
mPAP severity category, n (%)				<0.001
<25 mm Hg (normal)		41 (32)	253 (43)	
25–40 mm Hg (mild)		45 (35)	271 (46)	
41–55 mm Hg (moderate)		33 (25)	51 (9)	
>55 mm Hg (severe)		11 (8)	9 (2)	
Race, n (%)	3 (<1)			0.018
Caucasian		103 (79)	506 (87)	
African American		21 (16)	44 (8)	
Other		6 (5)	34 (6)	
Operative variables				
Ischemic time, min	22 (3)	329 ± 92.2	305 ± 92.3	0.008
Transplant type, single, n (%)	4 (1)	39 (30)	190 (33)	0.58
PRBC >1 L, n (%)	0 (0)	46 (35)	124 (21)	0.002
Cardiopulmonary bypass use, n (%)	4 (1)	78 (60)	196 (34)	<0.001

PGD is defined as Grade 3 PGD on Day 2 or 3 after lung transplantation. Continuous variables are listed as mean ± standard deviation. Percentages may not exactly equal 100% because of rounding.
mPAP, mean pulmonary arterial pressure; PRBC, packed red blood cells.

of lung epithelial injury pathways prior to transplantation leads to an increased susceptibility of PGD. Future investigation on the systemic immune effectors of these pathways in the posttransplant period is important.

We found that the association was the strongest in subjects without IPF as a pretransplant diagnosis. Overall, subjects with IPF had a significantly higher CC-16 level compared to other pretransplant diagnoses. The lack of association between CC-16 and PGD in IPF subjects may be that subjects with IPF already had such a strong signal of epithelial injury prior to transplantation that any subsequent injury related to PGD is difficult to detect as levels in IPF patients are so high (25). Alternatively, a recent study demonstrated that chronic obstructive pulmonary disease (COPD) patients with high levels of circulating inflammatory markers in a symptom-free period had a greater number of exacerbations (26). It may be that patients with COPD and other non-IPF diagnoses with high CC-16 levels are a subgroup of ''exacerbators'' that are at increased risk for

Table 2: Median (interquartile range) preoperative biomarker levels by PGD status

Biomarker	n	PGD (n = 130)	Non-PGD (n = 584)	p-Value
CC-16 (ng/mL)	712	10.1 (5.2, 19.0)	6.0 (3.4, 12.8)	<0.001
sRAGE (pg/mL)	712	743.3 (438.9, 2030.8)	725.6 (391.7, 1462.6)	0.27
ICAM-1 (ng/mL)	714	223 (135, 333)	214 (137, 340)	0.97
IL-8 (pg/mL)	714	5.5 (3.3, 12.9)	5.3 (3, 10)	0.36
Protein C (% control)	713	110 (77, 140)	105 (79, 134)	0.44

CC-16, club (Clara) cell secretory protein; ICAM-1, intercellular adhesion molecule-1; PGD, primary graft dysfunction; sRAGE, soluble receptor for advanced glycation end product.
p-Values were calculated using the Wilcoxon-rank sum test. The number of subjects with a valid biomarker measurement is listed next to the biomarker.

epithelial injury after transplant, and that relationship is washed out in IPF where there are consistently high CC-16 levels in all patients.

When added to a predictive model using clinical covariates, CC-16 only had a slight increase in utility for predicting PGD. Our findings indicate that preoperative CC-16 levels are independently associated with PGD, and worthy of further study into the mechanism of development of PGD in those subjects without IPF, although it has not been proven a useful predictor of those who will go on to develop PGD when measured preoperatively.

We were unable to demonstrate an association between Protein C, ICAM-1, IL-8 and sRAGE at the preoperative time point and PGD. Protein C, ICAM-1 and sRAGE have established associations with PGD at postoperative time point (5,7) and IL-8 is a marker of ALI (27); IL-8 was recently demonstrated to have good predictive utility for ALI when measured in the emergency department. Our inability to detect an association between these biomarkers and the subsequent development of PGD may be because these

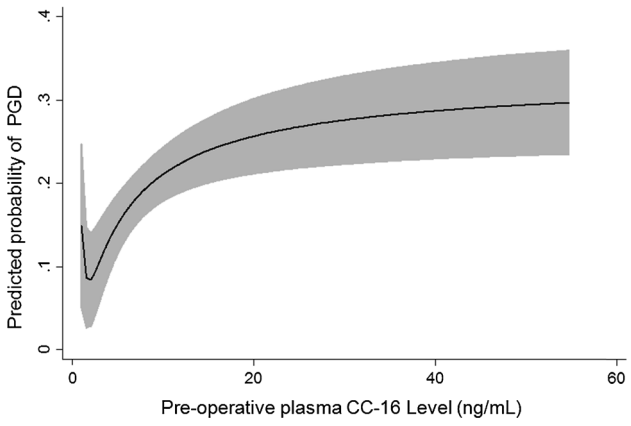

Figure 1: Relationship of CC-16 level to predicted probability of Grade 3 PGD on Day 2 or 3 using fractional polynomial plot. The gray area describes the 95% confidence interval. CC-16, club (Clara) cell secretory protein; PGD, primary graft dysfunction.

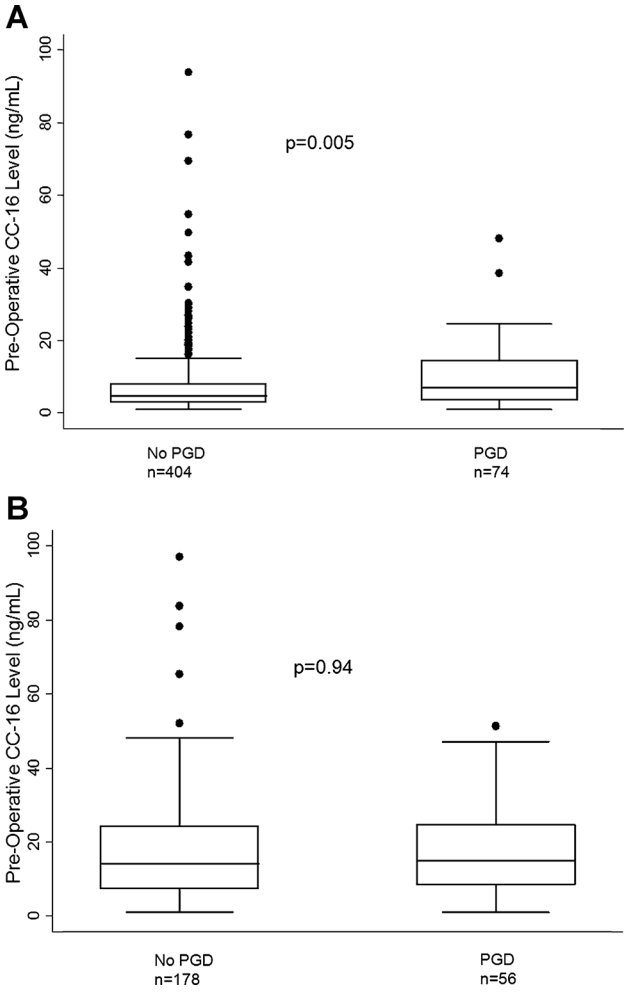

Figure 2: (A) CC-16 levels in those with PGD and those without PGD in subjects without idiopathic pulmonary fibrosis (IPF) as a pretransplant diagnosis. (B) CC-16 levels in those with PGD and those without PGD in subjects with IPF as a pretransplant diagnosis. The horizontal line in the middle of each box indicates the median; the top and bottom borders mark the 75th and 25th percentiles, respectively; and the whiskers mark the 90th and 10th percentiles. CC-16, club (Clara) cell secretory protein; PGD, primary graft dysfunction.

Table 3: Univariate and multivariate results of CC-16 association with PGD in subjects without IPF as a pretransplant diagnosis

Variable	Odds ratio for third quartile of CC-16	p-Value	Odds ratio for fourth quartile of CC-16	p-Value
CC-16	1.90 (0.97, 3.72)	0.06	2.87 (1.37, 6.01)	0.005
Adjusted for				
BMI	1.87 (0.95, 3.66)	0.07	2.83 (1.35, 5.94)	0.006
mPAP	1.64 (0.82, 3.30)	0.16	2.58 (1.20, 5.55)	0.015
Transplant type	2.05 (1.04, 4.05)	0.04	3.16 (1.49, 6.71)	0.003
Ischemic time	1.88 (0.96, 3.69)	0.07	2.85 (1.36, 5.97)	0.005
FiO_2 at reperfusion	1.95 (0.99, 3.84)	0.05	2.82 (1.34, 5.95)	0.006
Female sex and parity	1.99 (1.01, 3.92)	0.05	3.00 (1.42, 6.35)	0.004
PRBC	2.05 (1.04, 4.08)	0.04	3.34 (1.56, 7.08)	0.002
Donor Smoking	1.92 (0.98, 3.77)	0.06	2.90 (1.38, 6.07)	0.005
Cardiopulmonary bypass	1.68 (0.84, 3.33)	0.14	2.31 (1.08, 4.94)	0.031
Multivariable model	2.16 (1.00, 4.63)	0.049	3.03 (1.26, 7.30)	0.013

CC-16, club (Clara) cell secretory protein; FiO_2, fraction of inspired oxygen; IPF, idiopathic pulmonary fibrosis; mPAP, mean pulmonary arterial pressure; PGD, primary graft dysfunction; PRBC, packed red blood cell.
Multivariable model includes BMI, mPAP, transplant type, ischemic time, FiO_2 at reperfusion, female sex and parity, PRBC, donor smoking and cardiopulmonary bypass.

biomarkers reflect mechanisms that are activated by the process of ischemia-reperfusion injury, and not mechanisms that are active in the recipient prior to transplantation. Additionally, a small proportion of our preoperative biomarkers was measured at months prior to transplantation, and may have diluted our ability to detect an association using these biomarkers.

Our study has several limitations. First, not all of the plasma measurements were taken at the same time point, so there may have been other confounding factors associated with the earlier measurements. However, in sensitivity analyses, the association between CC-16 and PGD was unchanged in subjects who had plasma measurements at the time of listing or at the time of transplantation. This increases applicability of our findings, as the association was still present with biomarkers measured early; it supports the hypothesis that there may be time for potential interventions prior to transplantation. Second, we do not have available data on concomitant immunosuppressant medications at the time of preoperative blood draws. Although prior studies have successfully measured these markers in the setting of concomitant steroid use, little is known about the effect of immunosuppressants on human plasma levels; thus, residual confounding may account for some of our negative results (23,28–30). Third, we used multiple imputation to account for missing data in the covariates; however, missing data on clinical covariates were rare and we had no missing PGD grade and minimal missing biomarker measurements within the cohort.

In conclusion, we have demonstrated an association between preoperative levels of CC-16 and PGD in subjects without IPF as a pretransplant diagnosis. This finding sheds light on pretransplant, potentially modifiable factors that may lead to PGD. Further research is warranted focusing on the mechanisms of how recipient epithelial injury ''primes'' the lung for subsequent PGD.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Dr. Lederer consults for ImmuneWorks on lung transplantation and primary graft dysfunction. Dr. Wilkes is the co-founder and Chief Scientific Officer of ImmuneWorks. Drs. R. J. Shah, Palmer, Cantu, Flesch, Diamond, Kawut, Localio, Bellamy, Lama, Bhorade, Crespo, Sonnett, Wille, A. Shah, Weinacker, Arcasoy, P. Shah, Christie and Ware have no conflicts of interest.

References

1. Christie JD, Bavaria JE, Palevsky HI, et al. Primary graft failure following lung transplantation. Chest 1998; 114: 51–60.
2. Christie JD, Carby M, Bag R, 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 2005; 24: 1454–1459.
3. Christie JD, Kotloff RM, Ahya VN, et al. The effect of primary graft dysfunction on survival after lung transplantation. Am J Respir Crit Care Med 2005; 171: 1312–1316.
4. Christie JD, Kotloff RM, Pochettino A, et al. Clinical risk factors for primary graft failure following lung transplantation. Chest 2003; 124: 1232–1241.
5. Covarrubias M, Ware LB, Kawut SM, et al. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. Am J Transplant 2007; 7: 2573–2578.
6. Christie JD, Robinson N, Ware LB, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. Am J Respir Crit Care Med 2007; 175: 69–74.

7. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. Am J Respir Crit Care Med 2009; 180: 1010–1015.
8. Diamond JM, Kawut SM, Lederer DJ, et al. Elevated plasma Clara cell secretory protein concentration is associated with high-grade primary graft dysfunction. Am J Transplant 2011; 11: 561–567.
9. Shah RJ, Bellamy SL, Localio AR, et al. A panel of lung injury biomarkers enhances the definition of primary graft dysfunction (PGD) after lung transplantation. J Heart Lung Transplant 2012; 31: 942–949.
10. Diamond JM, Lee JC, Kawut SM, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med 2013; 187: 527–534.
11. Department of Health and Human Services HRaSA, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA. 2012. Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients. Transplant Data 1994–2012. 2012.
12. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. N Engl J Med 2011; 364: 1431–1440.
13. Lee JC, Christie JD. Primary graft dysfunction. Proc Am Thorac Soc 2009; 6: 39–46.
14. Hoffman SA, Wang L, Shah CV, et al. Plasma cytokines and chemokines in primary graft dysfunction post-lung transplantation. Am J Transplant 2009; 9: 389–396.
15. Hermans C, Petrek M, Kolek V, et al. Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis. Eur Respir J 2001; 18: 507–514.
16. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999; 28: 964–974.
17. Lederer DJ, Kawut SM, Wickersham N, et al. Obesity and primary graft dysfunction after lung transplantation: The Lung Transplant Outcomes Group Obesity Study. Am J Respir Crit Care Med 2011; 184: 1055–1061.
18. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993; 138: 923–936.
19. Shah RJ, Diamond JM, Cantu E, et al. Use of a simple prediction model improves pre-transplant risk stratification for primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med 2013; 187: A3773.
20. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: Potential and pitfalls. BMJ 2009; 338: 157–160.
21. Broeckaert F, Clippe A, Knoops B, Hermans C, Bernard A. Clara cell secretory protein (CC16): Features as a peripheral lung biomarker. Ann N Y Acad Sci 2000; 923: 68–77.
22. Janssen R, Sato H, Grutters JC, et al. Study of Clara cell 16, KL-6, and surfactant protein-D in serum as disease markers in pulmonary sarcoidosis. Chest 2003; 124: 2119–2125.
23. Lesur O, Langevin S, Berthiaume Y, et al. Outcome value of Clara cell protein in serum of patients with acute respiratory distress syndrome. Intensive Care Med 2006; 32: 1167–1174.
24. Kropski JA, Fremont RD, Calfee CS, Ware LB. Clara cell protein (CC16), a marker of lung epithelial injury, is decreased in plasma and pulmonary edema fluid from patients with acute lung injury. Chest 2009; 135: 1440–1447.
25. Sims MW, Beers MF, Ahya VN, et al. Effect of single vs bilateral lung transplantation on plasma surfactant protein D levels in idiopathic pulmonary fibrosis. Chest 2011; 140: 489–496.
26. Thomsen M, Ingebrigtsen TS, Marott JL, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. J Am Med Assoc 2013; 309: 2353–2361.
27. Pires-Neto RC, Morales MMB, Lancas T, et al. Expression of acute-phase cytokines, surfactant proteins, and epithelial apoptosis in small airways of human acute respiratory distress syndrome. J Crit Care 2013; 28: 111.e9–111.e15.
28. Seam N, Meduri GU, Wang H, et al. Effects of methylprednisolone infusion on markers of inflammation, coagulation, and angiogenesis in early acute respiratory distress syndrome. Crit Care Med 2012; 40: 495–501.
29. Jorens PG, Sibille Y, Goulding NJ, et al. Potential role of Clara cell protein, an endogenous phospholipase A2 inhibitor, in acute lung injury. Eur Respir J 1995; 8: 1647–1653.
30. Panwar R, Venkatesh B, Kruger P, et al. Plasma protein C levels in immunocompromised septic patients are significantly lower than immunocompetent septic patients: A prospective cohort study. J Hematol Oncol 2009; 2: 43.