



## Soluble P-Selectin and the Risk of Primary Graft Dysfunction After Lung Transplantation

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**Background:** Platelet activation with subsequent neutrophilic adherence to the vasculature initiates ischemia-reperfusion injury. We hypothesized that higher plasma P-selectin levels reflecting platelet activation would therefore be associated with primary graft dysfunction (PGD) after lung transplantation.

**Methods:** In a prospective, multicenter cohort study of 376 patients who had undergone lung transplantation between 2002 and 2007, we measured soluble P-selectin levels before lung transplantation and at 6 and 24 h after lung reperfusion in 20 patients with grade III PGD ( $\text{PaO}_2/\text{fraction of inspired oxygen}$ ,  $< 200$  mm Hg [with alveolar infiltrates seen on chest radiographs]) at 72 h after transplantation and 61 control subjects without PGD.

**Results:** Higher postoperative soluble P-selectin levels were associated with an increased risk of PGD at 72 h after transplantation (odds ratio [OR] per 1 natural log increase in soluble P-selectin at 6 h after lung allograft reperfusion, 3.5; 95% confidence interval [CI], 1.01 to 11.8;  $p = 0.048$ ) and at 24 h after lung allograft reperfusion (OR, 4.8; 95% CI, 1.4 to 16.1;  $p = 0.01$ ). Higher preoperative mean pulmonary artery pressure and the use of cardiopulmonary bypass were also associated with an increased risk of PGD.

**Conclusion:** Higher postoperative soluble P-selectin levels were associated with an increased risk of PGD at 72 h following lung transplantation. (CHEST 2009; 136:237–244)

**Abbreviations:** ALI = acute lung injury; CI = confidence interval; CPB = cardiopulmonary bypass;  $\text{FIO}_2$  = fraction of inspired oxygen; IQR = interquartile range; mPAP = mean pulmonary artery pressure; OR = odds ratio; PGD = primary graft dysfunction

Primary graft dysfunction (PGD) is a form of acute lung injury (ALI) occurring immediately after lung transplantation due to ischemia-reperfusion injury.<sup>1–4</sup> The incidence of PGD ranges from 10 to 25%, and PGD is the leading cause of early post-transplantation morbidity and mortality.<sup>1–3,5–7</sup> The risk factors for PGD are varied, involving the donor, recipient, and the processes of cold ischemia and reperfusion of the lung allografts.<sup>8,9</sup>

Prior studies<sup>10–13</sup> have demonstrated that the adhesion of neutrophils to the pulmonary vascular endothelium, diapedesis, and infiltration into the vessel wall are key initial steps in ALI and PGD, leading to the capillary leak that characterizes these syndromes. Activated platelets play an integral role in the tethering and

activation of neutrophils with eventual firm adherence to the vascular wall and transit to the interstitial and alveolar spaces. P-selectin is mobilized to the platelet surface from  $\alpha$  granules and to the endothelial cell membrane from Weibel-Palade bodies and serves as a receptor to a variety of ligands before being shed in the plasma in soluble form.<sup>14</sup> Although soluble P-selectin therefore may be of platelet or endothelial origin, it is considered a reliable and valid measure of platelet activation.<sup>15,16</sup> Animal models of ALI show elevations in soluble P-selectin levels,<sup>11</sup> suggesting that platelet activation is present. P-selectin may have mechanistic importance in PGD as well because P-selectin blockade (or P-selectin knockout) protected the lung allograft after ischemia and reperfusion in a mouse model.<sup>17</sup>

We have previously shown<sup>18</sup> that soluble P-selectin levels, CD40 ligand levels, and platelet-leukocyte aggregates (the most sensitive measure of platelet activation) increase in parallel after lung transplantation without cardiopulmonary bypass (CPB) compared to nontransplant thoracic surgery, indicating that soluble P-selectin in these patients is most likely shed from platelet sources in response to lung implantation. However, to our knowledge, no previous studies have addressed the relationship between platelet activity and PGD. In the current study, we hypothesized that platelet activation, as measured by increased soluble P-selectin levels, would be associated with a higher risk of PGD in lung transplantation recipients.

## MATERIALS AND METHODS

We performed a case-control study nested in a prospective cohort of 376 patients undergoing a first lung transplantation at seven centers in the United States (see Appendix for institutions and investigators). The study sample consisted of 20 randomly selected case patients with PGD grade III and 61 control subjects without PGD (PGD grade 0) enrolled between 2002 and 2007 with sufficient plasma. PGD case patients had diffuse alveolar infiltrates involving the lung allografts and, in the case of a single lung transplant, sparing the native lung, as seen on chest radiographs; PaO<sub>2</sub>/fraction of inspired oxygen (FIO<sub>2</sub>) ratio of < 200 mm Hg; and no other secondary cause of graft dysfunction identified, all at 72 h after transplantation. This definition has been validated previously.<sup>1,2,4,7</sup> Control subjects were patients from the cohort with clear lung allografts as determined by chest radiography (PGD grade 0) at 72 h after transplantation (see online supplement for details).

Informed consent for this study was obtained prior to organ transplantation. Blood samples were obtained in citrated tubes (Vacutainer; Becton Dickinson; Franklin Lakes, NJ) before trans-

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plantation, and at 6 h and 24 h after reperfusion of the lung allograft. Samples were centrifuged within 30 min of collection, and plasma samples were stored at -80°C. Clinical variables were categorized and defined using methods published previously.<sup>3</sup> Soluble P-selectin levels were measured in a central laboratory with an ultrasensitive, solid-phase sandwich, enzyme-linked immunosorbent assay (R&D Systems; Minneapolis, MN) [see online supplement for details].

## Statistical Analysis

Data were summarized as the mean  $\pm$  SD and median (interquartile range [IQR]), as appropriate. Unpaired *t* tests, rank sum tests, signed rank tests, and  $\chi^2$  or Fisher exact tests were used to compare PGD case patients with non-PGD control subjects. Correlations were assessed using Spearman rank correlation coefficients.

Bivariate and multivariate logistic regression models were used to assess the relationship between soluble P-selectin levels at baseline, 6 h, and 24 h postoperatively and the occurrence of PGD at 72 h after lung transplantation. Included in the multivariate analysis were potential confounding variables with *p* values < 0.20 in bivariate analyses. Potential confounders were included in the models one at a time; significant confounding was indicated by a change in the coefficient of the term for soluble P-selectin > 20%. Mean pulmonary artery pressure (mPAP) at anesthesia induction was considered in quartiles with indicator variables in the regression models; missing data were coded as such in this analysis. Soluble P-selectin levels were log transformed for the regression analysis. Power calculations are provided in the online supplement.

All statistical comparisons were performed using a statistical software package (Stata, version 10.0; Stata Corp; College Station, TX). This research protocol was approved by the institutional review boards at each of the participating centers.

## RESULTS

There were 81 patients in the study sample (Table 1). The mean age of the patients was  $53 \pm 12$  years, 33 patients (41%) were women, and 72 patients (89%) were non-Hispanic white. Most patients had undergone lung transplantation for COPD, and 25 patients (31%) received CPB.

Soluble P-selectin levels before lung transplantation and at 6 and 24 h after reperfusion of the lung allografts are shown in Figure 1. One patient had a missing preoperative P-selectin level, two had missing levels at 6 h, and one had a missing level at 24 h. In the entire study sample, median soluble P-selectin levels significantly increased from baseline (61 ng/mL [IQR, 47 to 80 ng/mL]) to 6 h (74 ng/mL [IQR, 50 to 111 ng/mL], *p* < 0.001 vs baseline) and to 24 h (69 ng/mL [IQR, 50 to 99 ng/mL], *p* = 0.003 vs baseline) after reperfusion.

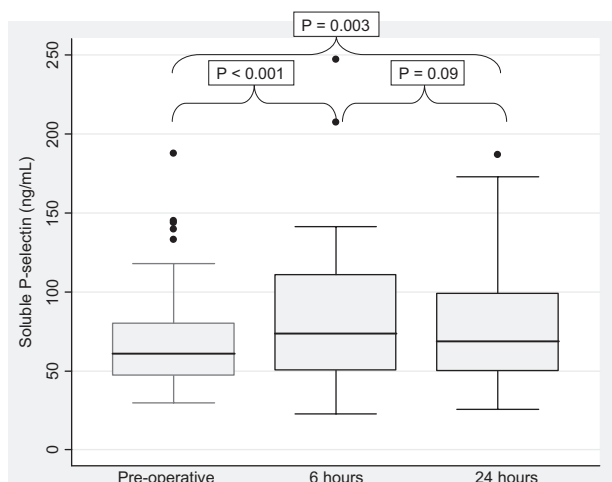
Among those PGD and non-PGD patients who did not receive CPB during the transplantation procedure (*n* = 56), there was still a significant median increase in soluble P-selectin levels from baseline (60 ng/mL [IQR, 46 to 76 ng/mL]) to 6 h (69 ng/mL [IQR, 45 to 104 ng/mL], *p* = 0.004 vs base-

**Table 1—Characteristics of the Study Sample**

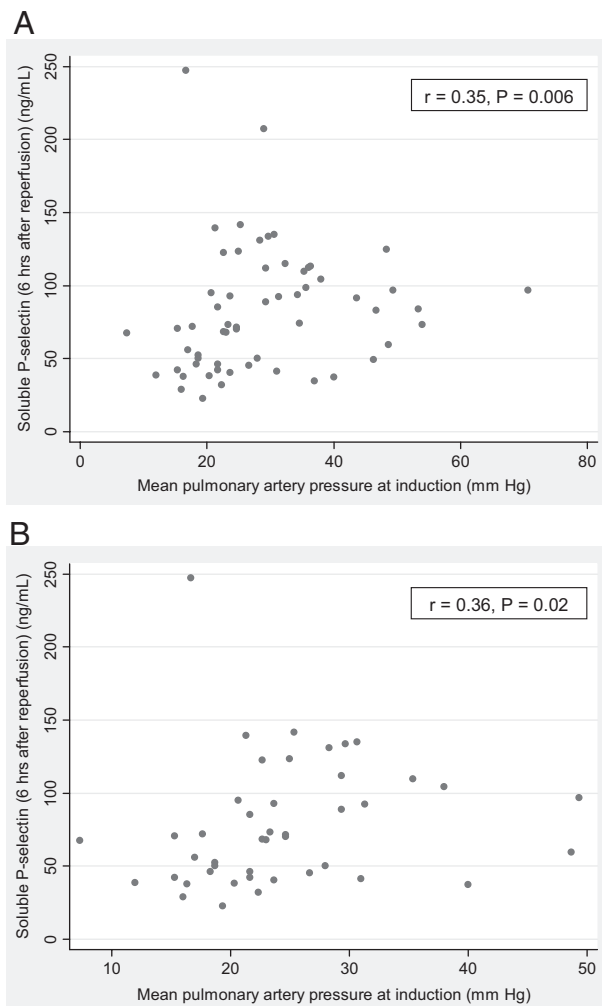
Characteristics	No.	Data
<b>Recipient variables</b>		
Age, yr	81	53 ± 12
Female sex	81	33 (41)
Race/ethnicity	81	
Non-Hispanic white		72 (89)
Black		8 (10)
Hispanic		1 (1)
Other		1 (1)
Diagnosis	81	
COPD		37 (46)
Diffuse parenchymal lung disease		28 (35)
Cystic fibrosis		10 (12)
Other		6 (7)
<b>Donor variables</b>		
Age, yr	81	31 ± 14
Female sex	81	34 (42)
Race/ethnicity	81	
Non-Hispanic white		61 (75)
Black		8 (10)
Hispanic		10 (12)
Other		2 (2)
<b>Surgical variables</b>		
Bilateral transplant	81	50 (62)
Use of CPB	81	25 (31)
mPAP at induction, mm Hg	61	29 ± 12
Ischemic time, min	77	293 (240–352)
Packed RBCs during first 24 h, mL	81	250 (0–1,000)
Platelets during first 24 h, mL	81	0 (0–0)
Fresh frozen plasma during first 24 h, mL	81	0 (0–450)

Values are given as the mean ± SD, No. (%), or median (IQR).

line) and possibly to 24 h (66 ng/mL [IQR, 45 to 95 ng/mL],  $p = 0.09$  vs baseline) after reperfusion. Focusing only on the non-PGD control group (with or without CPB) [ $n = 61$ ], we again found that median



**FIGURE 1.** Box-and-whisker plots of soluble P-selectin levels preoperatively and at 6 and 24 h after lung allograft reperfusion. Horizontal line = median; box = 25th and 75th percentiles; whiskers = adjacent values.



**FIGURE 2.** Scatterplots of soluble P-selectin levels at 6 h after lung allograft reperfusion vs mPAP at induction. *A*: all patients ( $n = 59$ ). *B*: patients not receiving CPB ( $n = 44$ ).

soluble P-selectin levels increased from baseline (60 ng/mL [IQR, 46 to 79 ng/mL]) to 6 h (70 ng/mL [IQR, 46 to 109 ng/mL],  $p = 0.002$  vs baseline) and possibly to 24 h (63 ng/mL [IQR, 45 to 97 ng/mL],  $p = 0.07$  vs baseline) after reperfusion.

Patients with diffuse parenchymal lung disease in the entire study sample may have had higher median soluble P-selectin levels before transplantation than patients with COPD (64 ng/mL [IQR, 59 to 87 ng/mL] vs 56 ng/mL [IQR, 38 to 69 ng/mL], respectively;  $p = 0.06$ ) Although mPAP at anesthesia induction for lung transplantation was not associated with soluble P-selectin levels at baseline in the entire study sample ( $r = 0.02$ ;  $p = 0.93$ ), there were significant direct associations between mPAP at anesthesia induction and soluble P-selectin levels at 6 and 24 h after lung transplantation in the entire study sample and in those who did not receive CPB (Fig 2, 3). In those patients who received CPB, there were no

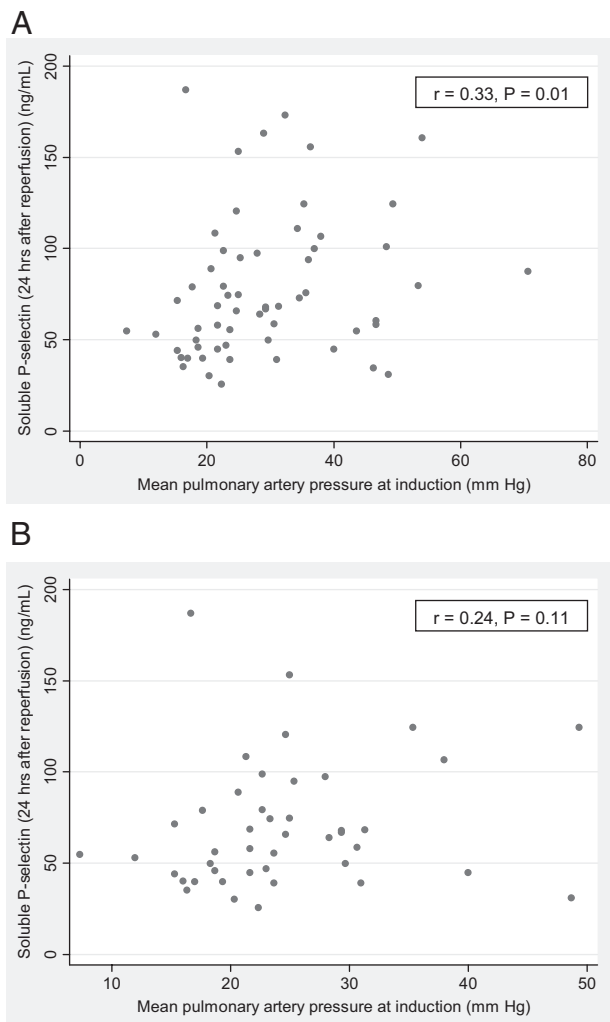


FIGURE 3. Scatterplots of soluble P-selectin levels at 24 h after lung allograft reperfusion vs mPAP at induction. A: all patients (n = 60). B: patients not receiving CPB (n = 44).

associations between CPB time and soluble P-selectin levels at any time point (data not shown).

We then compared PGD case patients to non-PGD control subjects (Table 2). PGD case patients tended to be younger than control subjects; however, there were no sex differences between the groups. PGD case patients were less likely to be non-Hispanic white and more likely to be black than control subjects. Case patients with PGD were more likely to have undergone transplantation for diffuse parenchymal lung disease than for COPD. PGD case patients had higher mPAP levels at induction, had more frequently used CPB, and had required transfusion of more blood products during transplantation and during the first 24 h postoperatively.

Case patients with PGD had median soluble P-selectin levels at baseline that were similar to those of control subjects without PGD (64 ng/mL [IQR, 56

to 83 ng/mL] vs 60 ng/mL [IQR, 46 to 79 ng/mL], respectively;  $p = 0.50$ ) [Fig 4]. However, case patients with PGD had higher median soluble P-selectin levels than non-PGD control subjects at 6 h (97 ng/mL [IQR, 73 to 112 ng/mL] vs 70 ng/mL [IQR, 46 to 109 ng/mL], respectively;  $p = 0.03$ ) and at 24 h (91 ng/mL [IQR, 74 to 118 ng/mL] vs 63 ng/mL [IQR, 45 to 97 ng/mL], respectively;  $p = 0.006$ ) after lung reperfusion.

Higher soluble P-selectin levels at 6 h after lung allograft reperfusion were associated with an increased risk of PGD (odds ratio [OR] per 1 natural log increase in soluble P-selectin level, 3.5; 95% confidence interval [CI], 1.01 to 11.8;  $p = 0.048$ ), as they were at 24 h after lung reperfusion (OR per 1 natural log increase in soluble P-selectin level, 4.8; 95% CI, 1.4 to 16.1;  $p = 0.01$ ) [Table 3]. Adjustment for a variety of variables, including sex, race, or pulmonary diagnosis, did not affect the results. Similarly, there was no change in the findings after adjustment for the use of blood products, such as packed RBCs, platelets, or fresh frozen plasma. The associations between soluble P-selectin levels and PGD case status seemed to be explained in part by the use of CPB because adjustment for this factor significantly reduced the effect estimates of soluble P-selectin levels.

## DISCUSSION

PGD contributes significantly to overall morbidity and is the major cause of early death following lung transplantation. We have shown that higher soluble P-selectin levels were associated with the occurrence of PGD in this case-control study nested within a large multicenter cohort of patients undergoing lung transplantation. Although increased mPAP and CPB are traditional risk factors for PGD (and were risk factors in our study), we have shown that increased soluble P-selectin levels and platelet activation may explain the mechanism of these associations. We have previously characterized PGD in terms of coagulation/fibrinolysis, endothelial dysfunction, inflammation, and oxidative stress.<sup>19–21</sup> However, these are the first data showing the importance of increased platelet activation in patients with PGD following human lung transplantation.

Platelets contribute to the course of ischemia-reperfusion injury in patients who have undergone transplants of other solid organs, such as the liver<sup>22</sup>; however, there is less known about this process in the lung.<sup>23</sup> Okada et al<sup>24</sup> showed that the accumulation of platelets in preserved and transplanted rat lungs was associated with the degree of reperfusion injury. Platelet adherence also resulted in capillary

**Table 2—Characteristics of PGD Case Patients and Control Subjects**

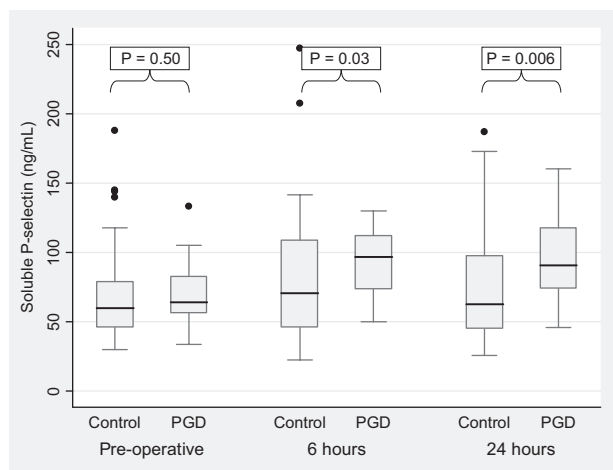
Characteristics	No.	Case Patients (n = 20)	Control Subjects (n = 61)	p Value
<b>Recipient variables</b>				
Age, yr	81	49 ± 14	54 ± 12	0.18
Female sex	81	8 (40)	25 (42)	0.94
Race/ethnicity	81			0.02
Non-Hispanic white		15 (75)	57 (93)	
Black		5 (25)	3 (5)	
Hispanic			1 (2)	
Diagnosis	81			0.03
COPD		5 (25)	32 (52)	
Diffuse parenchymal lung disease		12 (60)	16 (26)	
Cystic fibrosis		1 (5)	9 (15)	
Other		2 (10)	4 (7)	
<b>Donor variables</b>				
Age, yr	81	30 ± 13	32 ± 14	0.69
Female sex	81	8 (40)	25 (41)	0.94
Race/ethnicity	81			0.30
Non-Hispanic white		18 (90)	43 (71)	
Black			8 (13)	
Hispanic		2 (10)	8 (13)	
Other			2 (3)	
<b>Surgical variables</b>				
Bilateral transplant	81	13 (65)	37 (61)	0.73
Use of CPB	81	14 (70)	11 (18)	< 0.001
mPAP at induction, mm Hg	61	40 ± 14	25 ± 9	< 0.001
Ischemic time, min	77	286 (257–375)	300 (235–352)	0.50
Packed RBCs during first 24 h, mL	81	875 (0–1,500)	250 (0–750)	0.05
Platelets during first 24 h, mL	81	0 (0–622)	0 (0–0)	< 0.001
Fresh frozen plasma during first 24 h, mL	81	363 (0–1,053)	0 (0–0)	0.002

Values are given as the mean ± SD, No. (%), or median (IQR), unless otherwise indicated.

congestion. Other studies<sup>17,25</sup> of animal models of ischemia-reperfusion injury and ALI have not only documented platelet-mediated leukocyte infiltration in the lungs, but also specifically identified platelet-

derived P-selectin as the key molecule in this process. One study<sup>17</sup> utilized a rat model of orthotopic left lung transplantation after 6 h of hypothermic preservation. The administration of blocking anti-P-selectin antibody before reperfusion reduced pulmonary vascular resistance, increased PaO<sub>2</sub>, and improved survival. P-selectin knockout mice subjected to 30 min of pulmonary ischemia followed by reperfusion also showed significant reductions in neutrophil infiltration, increases in PaO<sub>2</sub>, and prolonged survival compared to wild-type mice. A model of lung ischemia-reperfusion in rabbits confirmed the rolling and adherence of platelets mediated by platelet P-selectin in the pulmonary vasculature after reperfusion.<sup>25</sup>

Zarbock et al<sup>26</sup> have shown that platelets play an important role in the accumulation of neutrophils in the intravascular, interstitial, and intraalveolar spaces of the lung in ALI. Platelet depletion in this model prevented abnormalities in gas exchange, accumulation of neutrophils in all three compartments, and protein leak. Antibodies against P-selectin prevented the formation of circulating platelet-neutrophil aggregates, decreased neutrophils in BAL fluid, improved the PaO<sub>2</sub>/FIO<sub>2</sub> ratio, and prolonged survival in this model. In addition, wild-type mice with ALI



**FIGURE 4.** Box-and-whisker plots of soluble P-selectin levels for PGD case patients and control subjects preoperatively and at 6 and 24 h after lung allograft reperfusion. Horizontal line = median; box = 25th and 75th percentiles; whiskers = adjacent values.

**Table 3—Logistic Regression Models for the Association of P-selectin Levels at 6 and 24 h With PGD**

Variables	OR for PGD per 1 ln Increase in P-selectin at 6 h After Reperfusion			OR for PGD per 1 ln Increase in P-selectin at 24 h After Reperfusion		
	95% CI	p Value		95% CI	p Value	
Unadjusted	3.5	1.01–11.8	0.048	4.8	1.4–16.1	0.01
Adjusted for						
Recipient age	2.9	0.8–10.9	0.11	4.4	1.2–16.0	0.02
Recipient sex	4.8	1.4–16.7	0.01	4.8	1.4–16.7	0.01
Recipient diagnosis	4.8	1.1–20.6	0.04	5.1	1.4–19.2	0.02
Recipient race/ethnicity	4.1	1.1–14.7	0.03	6.9	1.8–27.3	0.005
mPAP at induction	3.8	1.2–18.7	0.07	4.8	1.2–18.7	0.025
Bilateral transplant	3.5	1.0–12.0	0.05	4.9	1.4–17.1	0.01
Use of CPB	2.1	0.5–8.8	0.29	2.9	0.7–11.3	0.13
Ischemic time	3.2	0.9–11.5	0.07	4.2	1.2–14.8	0.03
Packed RBCs during first 24 h	3.7	1.0–13.2	0.04	5.1	1.5–17.6	0.01
Platelets during first 24 h	4.9	1.4–16.9	0.06	4.9	1.4–16.9	0.01
Fresh frozen plasma during first 24 h	2.9	0.8–10.4	0.10	4.5	1.3–15.6	0.02

ln = natural log.

and reconstituted with bone marrow from P-selectin knockout mice (thus permitting endothelial P-selectin but not megakaryocyte P-selectin expression) demonstrated better gas exchange and decreased neutrophil accumulation in the intravascular, interstitial, and intraalveolar spaces of the lung compared to wild-type mice with intact bone marrow. Another study<sup>27</sup> showed that platelet P-selectin was critical to the proinflammatory phenotype of endothelial dysfunction induced by high-tidal volume ventilation.

We have previously shown<sup>18</sup> that human lung transplantation itself is associated with platelet activation, even in the absence of traditional risk factors such as significant pulmonary hypertension and CPB. Our prospective cohort study<sup>18</sup> of patients undergoing lung transplantation without CPB and patients undergoing nontransplantation thoracic surgery showed significant increases in levels of soluble P-selectin, CD40 ligand, and circulating platelet-monocyte conjugates in the lung transplantation patients compared to the thoracic surgery patients. Interestingly, the increases in these platelet markers in the lung transplantation recipients were associated with decreases in platelet count, suggesting that more platelet activation was associated with greater platelet consumption or trapping, presumably in the lung allografts. These findings suggest that the transplantation procedure leads to platelet activation, even in the absence of CPB. Importantly, the persistence of the association between soluble P-selectin levels and PGD in the current study, despite adjustment for the use of blood products, indicates that transfusion does not account for our findings.

Colombat et al<sup>28</sup> have confirmed that platelet trapping occurs in the newly perfused lung allografts after human lung transplantation and has a signifi-

cant impact on oxygenation and allograft function. These investigators performed lung sampling 15 to 30 min after reperfusion. Platelet aggregation in the venular plexus was more prominent in the lung transplantation samples than in samples of lung tissue from control subjects. The finding of P-selectin-stained platelets in the vascular lumen after lung transplantation was associated with a longer duration of mechanical ventilation, lower PaO<sub>2</sub>/FIO<sub>2</sub> ratio, and pulmonary edema, as shown by chest radiography.

The association between pulmonary hypertension and PGD is complex and poorly understood. Some studies<sup>3</sup> have suggested that only the diagnosis of pulmonary arterial hypertension itself increases the risk of PGD; other studies<sup>29</sup> suggest that each higher increment of pulmonary artery pressure (from any underlying lung disease) translates to an increase in risk of PGD. In addition, because patients with significant pulmonary hypertension require CPB to undergo the transplantation procedure, it is difficult to tease out the respective causal roles. We showed that higher mPAP at induction was associated with higher soluble P-selectin levels both at 6 and 24 h after lung transplantation. This association appeared to persist even in those patients who did not require CPB, suggesting that preoperative pulmonary vascular disease results in platelet activation after lung transplantation independent of CPB. It is possible that long-standing pulmonary hypertension or right ventricular dysfunction may result in platelet defects leading to heightened susceptibility to an activated pulmonary vascular endothelium after transplantation. The complete lack of association between mPAP and soluble P-selectin levels at baseline suggests that some trigger is necessary to unmask this process.

There are several limitations to our study. First, PGD encompasses a spectrum of injury; we studied the extremes of this spectrum. Future prospective cohort studies should include patients with PGD grades I and II. Second, we intentionally did not match the control subjects to case patients in terms of pulmonary diagnosis or CPB use because such matching can introduce bias and actually decrease efficiency in certain situations. We did however adjust (or “control”) for differences in baseline covariates between case patients and control subjects in multivariate analyses, which is the preferable methodological approach in a study such as ours. Of course, the possibility of unmeasured or residual confounding exists.

In this study, we have provided the first evidence that elevated levels of soluble P-selectin are associated with PGD in human lung transplantation. These data and the strong body of evidence from animal models demonstrating that interference with P-selectin leads to attenuation of ischemia-reperfusion injury make targeting platelet activation a promising therapeutic approach in humans undergoing lung transplantation.

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## APPENDIX

### *Sites of and Participants in the Lung Transplant Outcomes Group*

*University of Alabama, Birmingham:* Keith Wille, MD; Joao de Andrade, MD; and Tonja Meadows, RN.

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*Stanford University:* Ann Weinacker, MD; Ramona Doyle, MD; Susan Spencer Jacobs, MSN; and Val Scott, MSN.

*Vanderbilt University:* Aaron Milstone, MD; Lorraine Ware, MD; E. Wesley Ely, MD, MPH; and Stacy Kelley-Blackburn, RN.

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