Association of Protein C and Type 1 Plasminogen Activator Inhibitor with Primary Graft Dysfunction

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Background: Acute lung injury is characterized by hypercoagulability and impaired fibrinolysis. We hypothesized that lower protein C and higher type 1 plasminogen activator inhibitor (PAI-1) levels in plasma would be associated with primary graft dysfunction (PGD) after lung transplantation.

Design: Prospective, multicenter cohort study.

Methods: We measured plasma levels of protein C and PAI-1 before lung transplantation and 6, 24, 48, and 72 h after allograft reperfusion in 128 lung transplant recipients at six centers. The primary outcome was grade 3 PGD ($\text{PaO}_2/\text{FiO}_2 < 200$ with alveolar infiltrates) 72 h after transplantation. Biomarker profiles were evaluated using logistic regression and generalized estimating equations.

Results: Patients who developed PGD had lower protein C levels 24 h posttransplantation than did patients without PGD (mean $\pm$ SD [relative to control]: 64 ± 27 vs. $92 \pm 41\%$, respectively; $p = 0.002$). Patients with PGD also had PAI-1 levels that were almost double those of patients without PGD at 24 h (213 ± 144 vs. 117 ± 89 ng/ml, respectively; $p < 0.001$). Throughout the 72-h postoperative period, protein C levels were significantly lower ($p = 0.007$) and PAI-1 levels were higher ($p = 0.026$) in subjects with PGD than in others. These differences persisted despite adjustment for potential confounders in multivariate analyses. Higher recipient pulmonary artery pressures, measured immediately pretransplantation, were associated with higher PAI-1 levels and increased risk of PGD.

Conclusion: Lower postoperative protein C and higher PAI-1 plasma levels are associated with PGD after lung transplantation. Impaired fibrinolysis and enhanced coagulation may be important in PGD pathogenesis.

Keywords: primary graft dysfunction; reperfusion injury; lung transplantation; type 1 plasminogen activator inhibitor; protein C

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

There is growing evidence that impaired fibrinolysis and increased coagulation play a role in acute lung injury pathogenesis. However, little is known about the role of coagulation and fibrinolysis in primary graft dysfunction in humans.

What This Study Adds to the Field

Elevation in plasma levels of PAI-1 and depression in protein C are associated with primary graft dysfunction.

Primary graft dysfunction (PGD) is a form of severe acute lung injury occurring after lung transplantation (1–4). The incidence of PGD ranges from 10 to 25%, and it is the leading cause of early posttransplantation morbidity and mortality (1–3, 5–9). The pathophysiology of PGD likely begins with brain death of the donor and progresses during organ ischemia, preservation, implantation, and reperfusion (10–12).

Coagulation and fibrinolytic abnormalities play a prominent role in the evolution of various forms of acute lung injury. Plasma levels of protein C drop while intravascular and intraalveolar fibrin deposition occur in patients with acute respiratory distress syndrome (ARDS) (13–15). On the other hand, levels of the fibrinolysis inhibitor type 1 plasminogen activator inhibitor (PAI-1) are increased in the plasma and pulmonary edema of patients with ARDS (16). Furthermore, administration of protein C reduces ischemia–reperfusion injury in experimental models (17, 18).

We hypothesized that enhanced coagulation, as measured by decreased protein C levels, and impaired fibrinolysis, as measured by increased plasma PAI-1 levels, would be associated with a higher risk of PGD in lung transplant recipients. We also aimed to elucidate the determinants of change in protein C and PAI-1 levels after lung transplantation.

METHODS

Study Population

We performed a prospective cohort study of patients undergoing first lung transplantation at six centers in the United States (see end of article for institutions and investigators). We excluded patients undergoing multiorgan transplantation. The study sample consisted of 128 lung

transplant recipients enrolled between June 2003 and November 2004 at these centers.

Definition of PGD

The primary outcome was International Society of Heart and Lung Transplantation (ISHLT) grade 3 PGD at 72 h, hereafter referred to as "PGD" (19). Briefly, patients with PGD had diffuse alveolar infiltrates involving the lung allograft(s) and, in the case of single lung transplant, sparing the native lung; $Pa_{O_2}/F_{I_{O_2}}$ less than 200 mm Hg; and no other secondary cause of graft dysfunction identified. This definition has been used in prior studies illustrating association with poor outcomes (1, 2, 7). Specific details of the PGD definition are included in the online supplement.

Data Collection and Management

Informed consent for this study was obtained before organ transplantation. Blood samples were obtained in citrated Vacutainers (Becton, Dickinson, and Company, Franklin Lakes, NJ) immediately before transplantation, and 6, 24, 48, and 72 h after reperfusion of the lung allograft(s). Samples were centrifuged within 30 min of collection and stored at -80°C . Clinical variables were categorized and defined according to methods published previously (3). Pulmonary hypertension was defined as a systolic pulmonary artery pressure (SPAP) > 45 mm Hg.

Methodology for Measurement of Protein C and PAI-1

Protein C was measured in plasma, using the Actichrome protein C assay (American Diagnostica, Greenwich, CT), and expressed as a percentage of control (20). PAI-1 antigen in plasma was measured with the Imubind plasma PAI-1 ELISA (American Diagnostica) and

expressed as nanograms per milliliter. All assays were performed in duplicate and average values were used in statistical analyses.

Statistical Analysis

Continuous variables were compared by t test or rank sum test. Correlations were assessed with Pearson or Spearman rank correlation coefficients. Contingency tables and χ^2 and Fisher exact tests were used, as appropriate. Generalized estimating equations (GEEs), a statistical tool to test relationships of longitudinal variables between groups, were used to assess the differences in biomarker profiles over time between PGD and non-PGD.

Multivariable logistic regression models were used to assess the potential confounding effects of clinical variables on the relationship between biomarker levels at 24 h and the occurrence of PGD. We included in the multivariable model potential confounding variables with $p < 0.20$ in bivariable analyses. One potential confounder was included in the model at a time. We also assessed the interaction of plasma biomarker levels with ischemic time and the use of cardiopulmonary bypass.

All statistical comparisons were performed with Stata version 8.0 (Stata Corp., College Station, TX), and SAS version 9.1 (SAS Institute, Cary, NC). This research protocol was approved by the institutional review boards at each of the participating centers.

RESULTS

The cohort included 128 transplant recipients. All had complete follow-up. The cumulative incidence of PGD at 72 h was 20% (95% confidence interval [CI]: 14, 28). Characteristics of the

TABLE 1. DONOR AND RECIPIENT CHARACTERISTICS BY PRIMARY GRAFT DYSFUNCTION STATUS

Variable	No PGD (<i>n</i> = 102)	PGD (<i>n</i> = 26)	p Value
Donor variables			
Age, yr: mean (95% CI)	30 (28, 33)	32 (25, 39)	0.54
Donor female sex, %	37	43	0.59
Donor race/ethnicity, %			
White	66	76	0.28
Black	21	5	
Hispanic	13	14	
Other	1	5	
Recipient and surgical variables			
Age, yr: mean (95% CI)	52 (49, 55)	45 (39, 53)	0.06
Female sex, %	47	31	0.11
Race/ethnicity, %			
White	90	81	0.06
Black	7	12	
Hispanic	2	0	
Asian	0	4	
Other	1	4	
Recipient diagnosis, %			0.01
Chronic obstructive pulmonary disease, %	58	19	
Diffuse parenchymal lung disease	26	50	
Cystic fibrosis	10	12	
Pulmonary arterial hypertension	1	8	
Congenital heart disease	5	11	
Other	1	0	
Procedure type, %			0.006
Single	54	20	
Bilateral	46	80	
Use of cardiopulmonary bypass, %	27	69	0.001
Pulmonary artery systolic pressure at induction: mean (95% CI)	40 (37, 43)	61 (45, 77)	< 0.001
Packed red blood cells, ml within 24 h post-transplantation: mean (95% CI)	421 (229, 613)	1,000 (601, 1,399)	0.008
Platelets, ml during first 24 h post-transplantation: mean (95% CI)	81 (35, 128)	329 (128, 529)	0.004
Fresh frozen plasma, ml during first 24 h post-transplantation: mean (95% CI)	230 (121, 336)	709 (296, 1,122)	0.001
Ischemic time			
First lung, min: mean (95% CI)	228 (215, 240)	241 (226, 256)	0.31
Second lung, min: mean (95% CI)	338 (309, 368)	341 (286, 396)	0.93

Definition of abbreviations: CI = confidence interval; PGD = primary graft dysfunction.

Continuous variables are expressed as means with 95% confidence intervals, and dichotomous variables as column percentages.

Figure 1. Protein C levels over time for patients with primary graft dysfunction (PGD) and patients without PGD. Means $\pm$ SE are shown. * $p < 0.05$ and ** $p < 0.01$ for PGD versus non-PGD.

study population are shown in Table 1. Younger and minority recipients tended to have a higher risk of PGD than did others. Patients with the diagnoses of pulmonary arterial hypertension and diffuse parenchymal lung disease had a higher risk of PGD. Patients with PGD more frequently underwent double (vs. single) lung transplantation, had higher SPAP at induction, received larger volumes of blood products, and were more likely to require cardiopulmonary bypass.

Protein C levels were lower in patients with PGD compared with others at each postoperative time point of the study ($p < 0.05$ at 6 h and $p < 0.01$ at each time point thereafter) (Figure 1). The GEE contrast estimate for this association was -18 (95% CI: $-32, -4.9$; $p = 0.007$), indicating that the protein C level was on average more than 18% lower over 72 h in patients who developed PGD. At 24 h posttransplantation, the protein C level was $64 \pm 27\%$ (relative to control) at 24 h in patients who subsequently developed PGD versus $92 \pm 41\%$ in those who did not (mean difference = 27% control [95% CI: 11, 45]; $p = 0.002$). However, preoperative levels did not differ between the two groups (108 ± 46 vs. $123 \pm 42\%$ control, respectively; $p = 0.24$).

Conversely, plasma PAI-1 levels rose dramatically in those who developed PGD, diverging from others at the 6-h time point to a maximum difference at 24 h before reconverging at later time points (Figure 2). The GEE contrast estimate was 43 (95% CI: 5, 81; $p = 0.026$), consistent with higher levels of PAI-1 over 72 h in those with PGD. The mean PAI-1 level at 24 h was 213 ± 144 ng/ml in those with subsequent PGD versus 117 ± 89 ng/ml in those without (mean difference, 97 ng/ml [95% CI: 53, 141]; $p < 0.001$). Preoperative levels of PAI-1 were similar between the two groups (94 ± 65 vs. 110 ± 111 ng/ml, respectively; $p = 0.56$).

To examine whether underlying differences in the recipients or surgical procedures explained these findings, we next assessed the association of 24-h biomarker levels with the risk of PGD after adjustment for potential confounders in multivariate analyses. The increase in risk (odds ratio) for PGD associated with each 50% decrease in protein C level at 24 h was 1.39 (95% CI: 1.14, 1.70; $p = 0.001$) (Table 2). There was no change in this odds ratio despite adjustment for a variety of clinical variables, including the use of cardiopulmonary bypass and volume of blood products administered. This confirms that the association

Figure 2. Type 1 plasminogen activator inhibitor (PAI-1) levels over time for patients with PGD and patients without PGD. Means $\pm$ SE are shown. * $p < 0.05$ and ** $p < 0.01$ for PGD versus non-PGD.

TABLE 2. MULTIVARIABLE ANALYSIS OF ASSOCIATION OF TYPE 1 PLASMINOGEN ACTIVATOR INHIBITOR AND PROTEIN C LEVELS AT 24 HOURS WITH PRIMARY GRAFT DYSFUNCTION

Variable	Protein C		PAI-1	
	Odds Ratio (95% CI)	p Value	Odds Ratio (95% CI)	p Value
Unadjusted base model	1.39 (1.14, 1.70)	0.001	1.44 (1.12, 1.76)	< 0.001
Adjusted for				
Recipient age	1.39 (1.14, 1.69)	0.001	1.43 (1.17, 1.74)	< 0.001
Recipient sex	1.27 (1.02, 1.59)	0.034	1.33 (1.07, 1.65)	0.010
Recipient diagnosis	1.37 (1.12, 1.68)	0.002	1.48 (1.19, 1.86)	0.001
Recipient race/ethnicity	1.42 (1.15, 1.75)	0.001	1.44 (1.18, 1.76)	< 0.001
Procedure type	1.36 (1.11, 1.67)	0.003	1.38 (1.12, 1.69)	0.001
Cardiopulmonary bypass	1.32 (1.08, 1.63)	0.008	1.39 (1.10, 1.77)	0.006
Total ischemic time	1.39 (1.13, 1.71)	0.002	1.51 (1.22, 1.87)	< 0.001
Pulmonary artery systolic pressure at transplantation	1.30 (1.06, 1.59)	0.028	1.23 (0.96, 1.59)	0.104
Blood (ml) during first 24 h	1.36 (1.11, 1.67)	0.003	1.40 (1.15, 1.72)	0.001
Platelet (ml) during first 24 h	1.35 (1.10, 1.66)	0.005	1.35 (1.10, 1.66)	0.005
Fresh frozen plasma (ml) during first 24 h	1.39 (1.13, 1.71)	0.002	1.37 (1.12, 1.69)	0.003

Definition of abbreviations: CI = confidence interval; PAI-1 = plasminogen activator inhibitor 1.

The reported odds ratios are for the relationship between change in each biomarker and primary graft dysfunction risk, adjusted individually for each confounder variable listed. Odds ratios for protein C are reported for each 10% control decrease in protein C, and for each 50-ng/ml increase in PAI-1.

between protein C level and PGD was independent of these other factors.

Similarly, the increase in risk of PGD associated with a 50-ng/ml increase in PAI-1 level at 24 h was 1.44 (95% CI: 1.12, 1.76; $p < 0.001$) (Table 2). This result was unchanged despite adjustment for clinical variables other than SPAP at the time of transplantation. There were no significant interactions between protein C or PAI-1 levels and ischemic time, the use of cardiopulmonary bypass, or the number of units of blood products (data not shown).

Given the attenuation of the association of PAI-1 and PGD with adjustment for preoperative SPAP, we next sought to investigate the potential relationship between SPAP and post-transplantation PAI-1 levels. Increased SPAP measured at induction was significantly associated with higher PAI-1 levels at 24 h (Figure 3) ($r = 0.46$, $p < 0.001$) but was not correlated with preoperative PAI-1 levels ($r = 0.10$, $p = 0.37$). Patients with pulmonary hypertension before transplantation had much higher PAI-1 levels both 6 and 24 h after transplantation. At 6 h, the mean plasma PAI-1 level among those with SPAP ≥ 45 mm Hg

was 231 ± 118 versus 175 ± 99 ng/ml among those with SPAP < 45 ($p = 0.011$). Likewise, at 24 h, mean PAI-1 was 188 ± 134 ng/ml among those with SPAP ≥ 45 mm Hg versus 109 ± 82 ng/ml ($p < 0.001$) (Figure 4). In contrast, preoperative levels of PAI-1 were not associated with pulmonary hypertension (115 ± 99 vs. 103 ± 106 ng/ml, $p = 0.62$). Adjustment for the use of cardiopulmonary bypass and other surgical variables did not change these results.

DISCUSSION

PGD contributes significantly to overall morbidity and is the major cause of early death after lung transplantation (1, 2, 7). We have shown that lower plasma levels of protein C after transplantation were associated with an increased risk of PGD independent of all other clinical variables. In addition, higher posttransplantation PAI-1 levels were associated with an increased risk of PGD. Although increased SPAP is a traditional clinical risk factor for PGD, we have shown that increased PAI-1 levels may mediate this association in part. These are the first

Figure 3. Systolic pulmonary artery pressure (SPAP) before transplantation at induction of anesthesia, plotted against plasma PAI-1 levels at 24 h. Solid triangles represent patients with PGD and open circles represent those with no PGD. The regression line is shown.

Figure 4. Plasma PAI-1 levels at 24 h in those with pulmonary hypertension at induction and those without pulmonary hypertension. Dots represent outliers.

data showing the importance of the coagulation/fibrinolytic pathways in PGD after human lung transplantation.

The early and persistent drop in protein C levels preceding PGD suggests that impaired production and/or increased consumption of protein C plays a role in the initiation and perpetuation of lung injury (13, 21, 22). This finding is consistent with previous studies showing that protein C levels are lower among subjects with acute lung injury (23). Notably, the protein C levels in the patients with PGD in the current study were similar to those of the patients with acute lung injury in this previous work. Lower protein C levels likely contribute to PGD in a similar fashion as they do to acute lung injury and ARDS, that is, by leading to greater microvascular thrombosis, intraalveolar activation of the coagulation cascade, and intraalveolar deposition of fibrin (13, 21, 23, 24).

Plasma PAI-1 levels increased greatly early after transplantation in patients with PGD, and then merged with others at the 48- and 72-h time points. Therefore, impaired fibrinolysis may be more pertinent to the earlier phases of posttransplantation lung injury, rather than its evolution.

Patients with severe pulmonary hypertension are known to have increased PAI-1 antigen levels and PAI-1 activity, which may be directly associated with pulmonary artery pressure (25–27). Although we did not find a relationship between SPAP and PAI-1 at baseline, there was a strong association with PAI-1 early after transplantation. In addition, our data suggest that increased PAI-1 may, in part, link pretransplantation pulmonary hypertension and the increased risk of PGD. The impaired fibrinolytic system that characterizes patients with pulmonary vascular disease may lead to a higher incidence of microthromboses in the lung allograft, resulting in an increased risk of PGD in these patients. Alternatively, elevated PAI-1 levels may just be an epiphenomenon of early PGD caused by the known risk factor of pulmonary hypertension.

There are several limitations to our study. First, PGD encompasses a spectrum of injury; we studied the extreme of this spectrum. However, it is this extreme form of PGD at 72 h that has a significant impact on morbidity and mortality of lung transplant recipients, justifying our focus (1, 7). Second, the possibility of unmeasured or residual confounding exists. We did not have information on preoperative use of anticoagulant medications or knowledge of inherent deficiencies in protein C.

However, our results were independent of the preoperative levels of protein C, making it unlikely that such baseline differences accounted for our findings. Finally, it is important to note that we measured protein C levels, and not activated protein C levels, as only the former assay was validated at the time our study was planned and executed. Future studies may focus on this and other novel measures of hypercoagulability and thrombolysis.

In this study, we have provided the first evidence that enhanced coagulation and impaired fibrinolysis are associated with PGD in the human lung transplant population. There is the possibility that these associations are epiphenomena of the syndrome. However, these findings suggest that coagulation/fibrinolytic pathways have an important role in PGD pathogenesis.

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