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Plasma Complement Levels Are Associated with Primary Graft Dysfunction and Mortality after Lung Transplantation

To the Editor:

Primary graft dysfunction (PGD) is a form of acute lung injury occurring within 72 hours of lung transplantation (1, 2). We have previously demonstrated an increase in early mortality in subjects
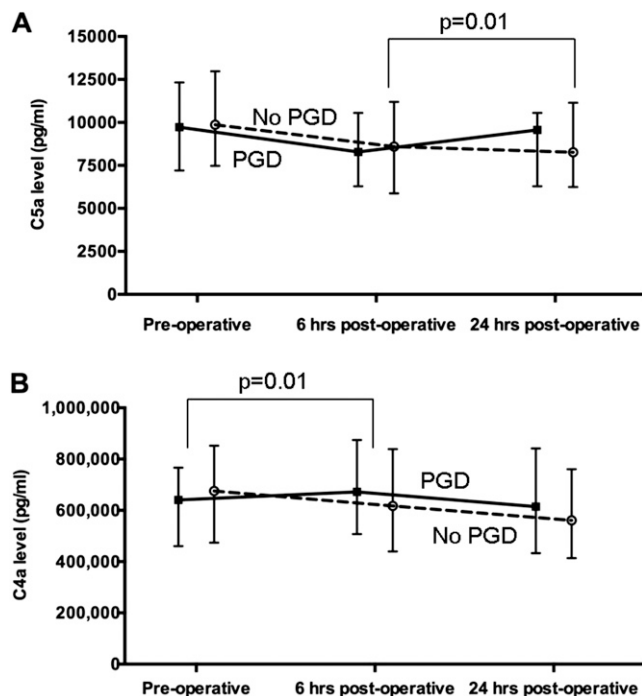

Figure 1. (*A*) Plasma C5a concentration at each time point in subjects without primary graft dysfunction (PGD) (*dashed line*) and with grade 3 PGD (*solid line*). The *P* value indicates that the difference in C5a concentration (represented by the slope of the line) between the 6-hour time point and the 24-hour time point is significantly greater in those with PGD compared with those without. (*B*) Plasma C4a concentration at each time point in subjects without PGD (*dashed line*) and with grade 3 PGD (*solid line*). The *P* value indicates that the difference in C4a concentration between the preoperative time point and the 6-hour time point (represented by the slope of the line) is significantly greater in those with PGD compared with those without.

with PGD (2). Understanding of the mechanisms leading to PGD and early mortality is important in identifying potential therapeutic targets and interventions.

The complement system defines a group of plasma and cell membrane proteins that play a key role in innate immunity as well as regulating adaptive immune response. C3a, C4a, and C5a, downstream products of the activated complement cascade, are potent neutrophil and lymphocyte chemoattractants (3–5). In animal models, activation of the complement system during lung ischemia and reperfusion can lead to cellular injury and lung allograft failure (6, 7).

Based on the relationship between complement activation and ischemia reperfusion injury in the lung allograft, we hypothesized that plasma levels of C3a, C4a, and C5a would be associated with both PGD and mortality after lung transplantation.

We performed a prospective cohort study of 190 lung transplant recipients from 10 centers enrolled between 2008 and 2010 in the Lung Transplant Outcomes Group (LTOG) cohort. We included consecutive subjects who had plasma samples available at the preoperative, 6-hour, and 24-hour time points (*see* Figure E1 in the online supplement). Clinical and mortality data were collected as described elsewhere (2). Informed consent was obtained from each subject. The Investigational Review Board at each center approved our study.

The primary outcome was grade 3 PGD within 72 hours after transplantation, which has demonstrated construct validity for survival (8). Grade 3 PGD at 24 hours was used to evaluate the relationship between concurrent lung injury, and grade 3 PGD at 48 or 72 hours was used to evaluate a severe lung injury phenotype (2). We evaluated all-cause mortality as an additional end point.

Plasma C3a, C4a, and C5a concentrations were measured using a commercially available cytometric bead array (BD Biosciences, San Jose, CA) in a manner devised to ensure stability of complement (*see* online supplement). We evaluated absolute differences in levels between each time point to assess changes during the early transplant period.

The association between complement and PGD was determined using Wilcoxon rank sum testing. We used Cox regression to evaluate associations between complement levels and time to death, conditioned on 90-day survival, to exclude bias from the effect of PGD and sepsis on early mortality. We adjusted for recipient, donor, and surgical variables (*see* online supplement).

Of the 190 subjects enrolled, 82 developed PGD (43%) within 72 hours post-transplant, and 33 had PGD at 48 or 72 hours (17%). Demographics of the cohort are in Table E1. There were no differences between subjects enrolled with blood samples at all three time points and other subjects enrolled in LTOG (Table E2).

The median change in plasma C5a levels between 6 and 24 hours post transplantation was significantly greater in subjects with PGD than in those without (867 vs. −156 pg/ml, $P = 0.01$) (Figure 1A). The median change in plasma C4a levels between preoperative and 6 hours was greater in subjects with PGD (72,075 vs. −79,501, $P = 0.01$) (Figure 1B). There was no significant difference in change in plasma C3a levels between the PGD and non-PGD group at any individual time point (Table E3). In sensitivity analyses, there was no difference in complement levels using grade 3 PGD at 24 hours (Table E4). However, there was a similar relationship between change in plasma C5a levels between 6 and 24 hours (1,164 vs.113, $P = 0.24$) and change in plasma C4a levels between preoperative and 6 hours (109,654 vs. −8,185, $P = 0.04$) using grade 3 PGD at 48 or 72 hours.

Of 175 patients with available mortality data, 32 died (17%). The change in C5a levels from 6 to 24 hours was associated with an increased risk of death (hazard ratio [HR], 1.81; 95% confidence interval [CI], 1.16, 2.83; $P = 0.01$). C5a levels measured preoperatively and 24 hours after transplantation were also associated with an increased risk of death (HR, 1.64; 95% CI, 1.29, 2.09; $P < 0.01$; and HR, 1.94; 95% CI, 1.39, 2.72; $P < 0.01$; respectively). C3a levels measured at 6 hours were associated with an increased risk of death (HR, 1.68; 95% CI, 1.04, 2.71; $P = 0.03$). There was no association between change in C3a or C4a levels and mortality (Tables E5 and E6). These associations were independent of adjustment for all tested confounders, including PGD (Table 1).

Our study demonstrates that early changes in plasma C4a and C5a are associated with PGD, and there is an association between C3a and C5a levels with mortality, independent of PGD. Collectively, these data suggest that systemic complement activation is important in both short- and long-term outcomes after transplantation.

Table 1. Association of C3a and C5a at Different Time Points with Mortality

Variable	HR C5a before Transplant (*n = 175*)	*P* Value	HR for C5a 24 h after Transplant (*n = 175*)	*P* Value	HR for Change in C5a between 6 and 24 h (*n = 171*)	*P* Value	HR for C3a at 6 h (*n = 159*)	*P* Value
Unadjusted (per 1 SD)	1.64 (1.29–2.09)	<0.01	1.81 (1.16–2.83)	<0.01	1.94 (1.39–2.72)	<0.01	1.68 (1.04–2.71)	0.03
Adjusted for								
Grade 3 PGD	1.65 (1.29–2.11)	<0.01	1.82 (1.17–2.95)	<0.01	1.95 (1.38–2.75)	<0.01	1.68 (1.04–2.70)	0.03
CBP	1.73 (1.28–2.28)	<0.01	1.86 (1.28–2.28)	<0.01	1.97 (1.39–2.80)	<0.01	1.71 (1.06–2.76)	0.03
Recipient race	1.63 (1.28–2.09)	<0.01	1.86 (1.20–2.89)	<0.01	1.93 (1.36–2.74)	<0.01	1.70 (1.04–2.77)	0.03
Recipient diagnosis	1.82 (1.33–2.49)	<0.01	1.92 (1.21–3.04)	<0.01	1.95 (1.36–2.80)	<0.01	1.73 (1.07–2.80)	0.03
Recipient sex	1.57 (1.23–1.99)	<0.01	1.79 (1.81–2.72)	<0.01	1.86 (1.34–2.59)	<0.01	1.71 (1.04–2.80)	0.03
Recipient age	1.95 (1.41–2.70)	<0.01	1.97 (1.26–3.09)	<0.01	2.00 (1.41–2.86)	<0.01	1.78 (1.09–2.89)	0.02
Donor sex	1.64 (1.28–2.10)	0.01	1.82 (1.16–2.88)	<0.01	1.94 (1.38–2.74)	<0.01	1.68 (1.04–2.72)	0.03
Donor age	1.64 (1.28–2.09)	<0.01	1.88 (1.22–2.91)	<0.01	1.94 (1.37–2.73)	<0.01	1.68 (1.04–2.73)	0.03
Donor race	1.66 (1.29–2.14)	<0.01	1.81 (1.15–2.83)	<0.01	2.02 (1.43–2.85)	<0.01	1.66 (1.00–2.75)	0.05
Donor smoking	1.69 (1.32–2.17)	<0.01	1.96 (1.27–3.04)	<0.01	2.06 (1.46–2.90)	<0.01	2.06 (1.46–2.90)	<0.01
PRBC	1.54 (1.18–2.01)	0.02	1.70 (1.10–2.67)	<0.01	1.68 (1.10–2.56)	0.02	1.63 (1.01–2.64)	0.05
Transplant type	1.66 (1.29–2.13)	<0.01	1.81 (1.16–2.82)	<0.01	1.94 (1.38–2.73)	<0.01	1.71 (1.05–2.77)	0.03
PASP	1.64 (1.27–2.11)	0.02	1.79 (1.11–2.89)	<0.01	1.99 (1.37–2.90)	<0.01	1.74 (1.03–2.95)	0.04

Definition of abbreviations: HR = hazard ratio; PASP = pulmonary artery systolic pressure at the time of transplantation; PGD = primary graft dysfunction within 72 h; PRBC = packed red blood cell transfusion within 24 h.
HRs with 95% confidence intervals are presented per 1-SD change in complement level.

The increase in C5a between 6 and 24 hours is associated with both PGD and mortality. This suggests a role for an early systemic complement response in both early and late outcomes. Prior work has implicated complement activation in lung ischemia–reperfusion injury (9). The change in C5a level was observed early after transplant and was not associated with concurrent lung injury (grade 3 at 24 h), indicating it may be driving lung injury and not just a marker of injury. Complement may be important in the pathogenesis PGD by attracting neutrophils and accelerating both the innate and adaptive immune responses within the first 24 hours after injury (10, 11).

The association of complement with mortality was independent of PGD. Prior work found an association between C3a, C5a, and obliterative bronchiolitis in a mouse model (12). Our findings in humans suggest a role for complement activation that continues after the early reperfusion period, potentially a signal of chronic immune activation starting in the early post-transplant period and leading to formation of autoantibodies, leading to chronic graft dysfunction and mortality. These findings support the study of blockade of complement activation to improve clinical outcomes after transplantation.

Our study had limitations. We were unable to measure complement levels in bronchoalveolar lavage; therefore, we could not link the plasma and lung compartments. The incidence of PGD in this study was 40%, which is higher than previous reports; however, we observed a similar trend in sensitivity analyses using a more restrictive definition of PGD. Finally, we were unable to adjust for center effect given small numbers.

In conclusion, our study suggests a role for early, systemic activation of the complement pathway in development of severe PGD and mortality after lung transplantation. ∎

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The Hemodynamic Effects of Prone Positioning in Patients with Acute Respiratory Distress Syndrome Remain to Be Defined

To the Editor:

We have a number of concerns regarding the results and conclusions of the study by Jozwiak and colleagues (1) and the accompanying editorial by Magder (2).

Jozwiak and colleagues' Table 2 indicates that the hemodynamic data were obtained with patients in the supine and prone positions, but the Methods section implies that the "supine" measurements were made with patients in the 45° upright posture. We would like clarification as to which posture was used as the "control." Zero reference pressures, central vascular filling pressures, and bladder pressure will change when moving from a 45° upright to 0° supine or prone. Accordingly, the changes the authors and editorialist attribute to prone positioning could have been due to taking patients out of the 45° upright position rather than to turning them prone *per se*. The effect of passive leg raising will also be affected by whether the patients were supine or in the 45° upright posture.

The level of positive end-expiratory pressure was changed between the hemodynamic measurements obtained in the two postures. Although the mean change was only 2 cm H_2O, even such a small difference can affect venous return, left- and right-sided transmural pressures, mean systemic venous pressure, and, to some extent, transpulmonary pressure.

Sixteen of the 18 patients were receiving inhaled NO. Although the dose was kept constant during the study, regional ventilation most definitely changes with prone positioning, and this will increase the number of vessels that might be exposed to NO, thereby altering global pulmonary vascular resistance.

Patients whose cardiac index did not change with prone positioning had a mean cardiac index (interquartile range) measured in the supine position (or 45° upright?) of 3.2 (2.8, 3.6) L/min/m^2, yet the average heart rate of patients in this group was reported as being 104 beats/min and the average stroke volume as 38 ml/m^2. Based on these two measurements, the average cardiac index should have approximated 3.95 L/min/m^2. This discrepancy is neither noted nor explained, and is particularly important in light of the reported increase in the mixed venous oxygen saturation (from 71 to 77%) that occurred in this group of patients on turning to the prone position. How could this occur if cardiac index did not change?

Although a number of clinical and laboratory studies have shown that prone positioning rarely, if ever, has adverse hemodynamic effects, we suggest that problems with the methodology used by Jozwiak and colleagues (1), and the results they present, do not allow us to conclude that prone positioning benefits hemodynamics to any meaningful extent or to accurately assess the effects of preload as they describe. ∎

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Reply: Prone Positioning Actually Exerts Benefits on Hemodynamics!

From the Authors:

We read with interest the comments of Drs. Albert and Hubmayr about our study (1). Concerning the first of their comments, patients were in the 45° semirecumbent position at baseline (2) as stated in the Methods. We agree that the hemodynamic effects of prone positioning should result from the addition of lowering the trunk to the horizontal position and prone positioning from the supine position, as we have previously reported (3) and noted in the Discussion. The postural change we used in the present study is recommended for clinical practice and was the method used in recent trials (4) (i.e., starting from the semirecumbent position).

Pressure transducers were fixed directly on the patient's thorax and were not moved from this position during the postural changes. Pressure transducers were carefully zeroed against atmospheric pressure after each postural change. Finally, we agree that the hemodynamic effects of passive leg raising are influenced by the starting position of the patient, as we have also previously reported (3). This is particularly relevant to our study, in which prone positioning also started from the semirecumbent position (2).

The adjustment of positive end-expiratory pressure after prone positioning resulted only in a 2-cm H_2O (1.5 mm Hg) increase.