Plasma Levels of Receptor for Advanced Glycation End Products, Blood Transfusion, and Risk of Primary Graft Dysfunction

Jason D. Christie^{1,2}, Chirag V. Shah^{1,2}, Steven M. Kawut^{1,2,3}, Nilam Mangalmurti¹, David J. Lederer⁴, Joshua R. Sonett⁵, Vivek N. Ahya¹, Scott M. Palmer⁶, Keith Wille⁷, Vibha Lama⁸, Pali D. Shah⁹, Ashish Shah⁹, Ann Weinacker¹⁰, Clifford S. Deutschman¹¹, Benjamin A. Kohl¹¹, Ejigayehu Demissie^{1,2}, Scarlett Bellamy², and Lorraine B. Ware¹², for the Lung Transplant Outcomes Group*

¹Pulmonary, Allergy, and Critical Care Division, ²Department of Biostatistics and Epidemiology, ³Penn Cardiovascular Institute, and ¹¹Department of Anesthesia and Critical Care, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; ⁴Division of Pulmonary, Allergy, and Critical Care Medicine, and ⁵Department of Surgery, Columbia University College of Physicians and Surgeons, New York, New York; ⁶Division of Pulmonary, Allergy, and Critical Care Medicine, Duke University, Raleigh-Durham, North Carolina; ⁷Division of Pulmonary and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, Alabama; ⁸Division of Pulmonary, Allergy, and Critical Care Medicine, University of Michigan, Ann Arbor, Michigan; ⁹Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Johns Hopkins University Hospital, Baltimore, Maryland; ¹⁰Division of Pulmonary and Critical Care Medicine, Stanford University, Palo Alto, California; and ¹²Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University, Nashville, Tennessee

Rationale: The receptor for advanced glycation end products (RAGE) is an important marker of lung epithelial injury and may be associated with impaired alveolar fluid clearance. We hypothesized that patients with primary graft dysfunction (PGD) after lung transplantation would have higher RAGE levels in plasma than patients without PGD.

Objectives: To test the association of soluble RAGE (sRAGE) levels with PGD in a prospective, multicenter cohort study.

Methods: We measured plasma levels of sRAGE at 6 and 24 hours after allograft reperfusion in 317 lung transplant recipients at seven centers. The primary outcome was grade 3 PGD ($\text{PaO}_2/\text{FiO}_2 < 200$ with alveolar infiltrates) within the first 72 hours after transplantation.

Measurements and Main Results: Patients who developed PGD had higher levels of sRAGE than patients without PGD at both 6 hours (median 9.3 ng/ml vs. 7.5 ng/ml, respectively; $P = 0.028$) and at 24 hours post-transplantation (median 4.3 ng/ml vs. 1.9 ng/ml, respectively; $P < 0.001$). Multivariable logistic regression analyses indicated that the relationship between levels of sRAGE and PGD was attenuated by elevated right heart pressures and by the use of cardiopulmonary bypass. Median sRAGE levels were higher in subjects with cardiopulmonary bypass at both 6 hours ($P = 0.003$) and 24 hours ($P < 0.001$). sRAGE levels at 6 hours were significantly associated with intraoperative red cell transfusion (Spearman's $\rho = 0.39$, $P = 0.002$ in those with PGD), and in multivariable linear regression analyses this association was independent of confounding variables ($P = 0.02$).

Conclusions: Elevated plasma levels of sRAGE are associated with PGD after lung transplantation. Furthermore, plasma sRAGE levels are associated with blood product transfusion and use of cardiopulmonary bypass.

Keywords: primary graft dysfunction; reperfusion injury; lung transplantation; receptor for advanced glycation end products; acute lung injury

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* A complete list of members may be found at the end of the article.

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Correspondence and requests for reprints should be addressed to Jason D. Christie, M.D., M.S., Assistant Professor of Medicine and Epidemiology, Division of Pulmonary, Allergy, and Critical Care Medicine, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, 423 Guardian Drive, 719 Blockley Hall, Philadelphia, PA 19104. E-mail: jchristi@mail.med.upenn.edu

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Soluble receptor for advanced glycation end products (sRAGE) is a novel marker of alveolar epithelial injury, and RAGE blockade attenuates experimental lung ischemia reperfusion injury. The role of sRAGE in clinical primary graft dysfunction is incompletely understood.

What This Study Adds to the Field

This multicenter cohort study demonstrates that sRAGE is elevated in primary graft dysfunction, suggesting that RAGE blockade may be a useful future therapy. In addition, sRAGE was elevated with use of cardiopulmonary bypass and red cell transfusion.

Primary graft dysfunction (PGD) is severe acute lung injury after lung transplantation (1–4). The pathophysiology of severe PGD is similar to other forms of acute lung injury (5) and represents the leading cause of early post-transplantation morbidity and mortality (1–3, 6–10). In humans, biomarkers of impaired coagulation and fibrinolysis (5), endothelial cell dysfunction (11), chemotaxis (7, 12), and cell adhesion (13) have been associated with PGD. However, the role of alveolar epithelial injury in PGD has not been fully evaluated.

The receptor for advanced glycation end products (RAGE) is a ubiquitously expressed receptor for nonenzymatically glycated adducts of endogenous proteins, lipids, and nucleic acids. These adducts form in the presence of hyperglycemia or oxidant stress and are termed advanced glycation end products (AGE). RAGE also binds to proinflammatory molecules, including S100 and high mobility group box 1 (14). Although RAGE is expressed in systemic vascular endothelium and nervous tissues, it is also highly localized to the lung where it is abundant in the basolateral membrane of alveolar epithelial type I cells (15). Recently, soluble RAGE (sRAGE) has been recognized as a potential plasma marker of type 1 cell injury. In both experimental and human acute lung injury, sRAGE is released into the alveolar and plasma compartments (16). A previous study in lung transplant recipients reported that plasma levels of sRAGE were associated with longer post-

operative ICU stays; however, this investigation lacked sufficient power to detect an association between plasma sRAGE and clinical PGD (17).

We hypothesized that elevated plasma levels of RAGE within the first 24 hours post-transplant would be associated with the subsequent development of PGD. We also aimed to examine potential determinants of elevations in plasma sRAGE levels after lung transplantation.

METHODS

Study Population

We undertook a prospective cohort study of patients undergoing first lung transplantation at seven centers in the United States (5, 13). The sample for the current study consisted of 317 lung transplant recipients enrolled between June 2003 and February 2007 at these centers. Plasma samples were obtained in citrated tubes at 6 and 24 hours after reperfusion of the lung allograft(s). Samples were centrifuged within 30 minutes of collection and stored at -80°C . Clinical variables were categorized and defined using methods published previously (3). Use of blood and blood products was defined as only those products administered during the surgical procedure. Right heart catheterization was performed at induction of anesthesia. The study was approved by the Institutional Review Boards at each of the participating centers. Informed consent was obtained from all participants.

Definition of Primary Graft Dysfunction

The primary outcome was PGD, defined as the International Society of Heart and Lung Transplantation grade 3 PGD on postoperative days 1, 2, or 3 (T24, T48, or T72) (18). As per the International Society of Heart and Lung Transplantation definition, PGD cases had diffuse alveolar infiltrates involving the lung allograft(s) that spared the native lung in the case of single lung transplantation; $\text{PaO}_2/\text{FiO}_2$ less than 200 mm Hg; and no other secondary cause of graft dysfunction, as defined in detail in prior publications (2, 3, 5, 13, 18). This definition of “any grade 3 PGD” has been adopted in a number of recent investigations (19–23). In addition, we conducted sensitivity analyses using patients with grade 3 PGD present at 72 hours, as in prior studies (3, 5, 8, 13).

Laboratory Methods for sRAGE Measurements

Levels of sRAGE were measured in thawed plasma samples in duplicate by sandwich ELISA (R&D, Minneapolis, MN) compared with a standard curve of recombinant protein provided by the manufacturer. The intraassay coefficient of variation was 5.9%. Personnel responsible for performing assays had no knowledge of clinical data.

Statistical Analysis

Continuous variables were compared using rank sum tests, and correlations were assessed using Spearman rank correlation coefficients. Contingency tables, Chi-square and Fisher exact tests were used, as appropriate. Multivariable logistic regression models were used to assess the potential confounding effects of clinical variables on the association of sRAGE levels at both 6 and 24 hours and the occurrence of PGD. sRAGE was assessed for proportionality of association with odds of PGD by plotting quartiles of sRAGE against PGD. In logistic regression equations, sRAGE was expressed as OR per 10-ng/ml increment (as this approximated the standard deviation of sRAGE). Potential confounders were included in the model one at a time to assess the confounding effects of each variable on the relationship of sRAGE (24). Linear regression was used to assess the relationship of continuous variables with sRAGE (such as units of blood transfused). In all linear regression equations, sRAGE was log transformed. Summary multivariable models included multiple potential confounding variables simultaneously if the confounders changed the coefficient by 15% or greater with adjustment (24). In addition, we examined the interaction of sRAGE levels with transplant procedure type and the use of cardiopulmonary bypass, in *a priori* specified analyses using multiplicative interaction terms in the logistic models. All statistical comparisons were performed using STATA version 10.0 (STATA Corp., College Station, TX).

RESULTS

Association of sRAGE with PGD

The study sample included 317 lung transplant recipients. The incidence of PGD at any time point during the first 3 days after transplant was 27% (95% CI, 22–32%). The prevalence of PGD at 24 hours after transplant was 20% (95% CI, 16–25%), at 48 hours was 14% (95% CI, 10–19%), and at 72 hours was 14% (95% CI, 10–18%). Characteristics of the donor and recipient study population by PGD status are presented in Table 1. Recipients with PGD were more likely to be African American than those without PGD. Patients with PGD more commonly had diffuse parenchymal lung disease (pulmonary fibrosis) or pulmonary arterial hypertension and were less likely to have chronic obstructive pulmonary disease. Subjects with PGD had higher pulmonary arterial pressures at the induction of anesthesia. Use of cardiopulmonary bypass and blood products was more common in the patients with PGD.

Overall, plasma sRAGE levels were elevated in the immediate postoperative period and then decreased at 24 hours: median 7.9 ng/ml (interquartile range [IQR]: 3.8, 32.4) at 6 hours versus 2.3 ng/ml (IQR: 1.4, 4.5) at 24 hours after reperfusion ($P < 0.001$). sRAGE levels were significantly higher in patients with PGD compared with others at both 6 and 24 hours postoperatively (Figure 1). The median 6-hour sRAGE level was 9.3 ng/ml (IQR: 4.7, 19.7) in those with PGD versus 7.5 ng/ml

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

Donor Variables	No PGD N = 233	PGD N = 84	P Value
Age, yr	33 (31, 35)	35 (32, 39)	0.19
Female sex	40%	46%	0.29
Race/ethnicity			0.24
White	68%	74%	
African American	20%	11%	
Hispanic	10%	11%	
Other	2%	4%	
Recipient and surgical variables			
Age, yr	52 (51, 54)	46 (46, 53)	0.10
Female sex	48%	49%	0.85
Race/ethnicity			0.03
White	91%	77%	
African American	5%	18%	
Hispanic	2%	2%	
Other	2%	2%	
Recipient diagnosis			<0.001
Chronic obstructive pulmonary disease	53%	32%	
Diffuse parenchymal lung disease	28%	40%	
Cystic fibrosis	11%	8%	
Pulmonary arterial hypertension	2%	7%	
Sarcoidosis	1%	7%	
Congenital heart disease	3%	2%	
Other	2%	2%	
Procedure type: single	42%	32%	0.46
Use of cardiopulmonary bypass	27%	55%	<0.001
Pulmonary artery systolic pressure at induction, mm Hg	40 (37, 42)	52 (46, 59)	<0.001
Packed red blood cells transfused, ml	507 (399, 614)	727 (556, 899)	0.03
Platelets transfused, ml	127 (53, 201)	452 (455, 659)	0.003
Ischemic time, min			
First lung	227 (219, 234)	242 (226, 258)	0.06
Second lung	328 (313, 342)	316 (292, 340)	0.41

Definition of abbreviation: PGD = primary graft dysfunction.

PGD defined as any grade 3 PGD during first 72 hours. Continuous variables are expressed as means with 95% confidence intervals, and dichotomous variables as column percentages, which may not exactly total 100% because of rounding.

Figure 1. Box and whisker plots of plasma receptor for advanced glycation end products (RAGE) levels at (A) 6 hours, and (B) 24 hours in patients with primary graft dysfunction (PGD) ($n = 84$) and patients without PGD ($n = 233$). Boxes display medians with interquartile ranges; error bars indicate 10th to 90th percentiles.

(IQR: 3.8, 13.5) in those without ($P = 0.028$). Median sRAGE level at 24 hours post-transplant was 4.3 ng/ml (IQR: 2.6, 9.3) in those with PGD versus 1.9 ng/ml (IQR: 1.0, 3.8) in those without ($P < 0.001$).

We next assessed the association of sRAGE levels with PGD after adjustment for potential confounding variables in multivariable analyses. The unadjusted odds ratio (OR) for PGD associated with a 10-ng/ml increase in sRAGE level at 6 hours after reperfusion was 1.28 (95% CI, 1.10–1.49; $P = 0.002$). Adjustment for pulmonary artery systolic pressure in the model attenuated the relationship between the 6-hour sRAGE level and subsequent PGD (Table 2). Inclusion of additional covariates to the model did not change this result. There was no interaction (effect modification) between use of cardiopulmonary bypass ($P = 0.64$) or transplant procedure type ($P = 0.99$) and the relationship of sRAGE levels at 6 hours with PGD. Results were similar in examining 24-hour sRAGE level and PGD risk. The unadjusted OR for each 10-ng/ml increase in sRAGE at 24 hours was 1.46 (95% CI, 1.01–2.12; $P = 0.043$). There was no confounding by clinical variables except for use of cardiopulmonary bypass (adjusted OR, 1.29; 95% CI, 0.93–1.80; $P = 0.14$), and pulmonary artery systolic pressure (adjusted OR, 1.27; 95% CI, 0.93–1.73; $P = 0.13$).

Results were unchanged when the definition of PGD was restricted to grade 3 PGD present at 72 hours. Using this definition in the total population ($n = 317$), the unadjusted OR

TABLE 2. MULTIVARIABLE ANALYSES OF ASSOCIATION OF SOLUBLE RAGE LEVELS AT 6 HOURS POSTREPERFUSION AND PRIMARY GRAFT DYSFUNCTION (N = 317)

Variable	Odds Ratio per 10-ng/ml Increment in sRAGE (95% CI)	P Value
Unadjusted	1.28 (1.10–1.49)	0.002
Adjusted for		
Recipient age	1.27 (1.08–1.49)	0.004
Recipient sex	1.28 (1.10–1.49)	0.002
Recipient diagnosis	1.32 (1.11–1.57)	0.001
Recipient race/ethnicity	1.27 (1.08–1.49)	0.003
Procedure type	1.29 (1.10–1.51)	0.002
Cardiopulmonary bypass	1.20 (1.02–1.41)	0.025
Ischemic time	1.29 (1.09–1.53)	0.003
Pulmonary artery systolic pressure at transplant	1.18 (0.93–1.49)	0.16
Packed red blood cell transfused	1.25 (1.06–1.47)	0.007

Definition of abbreviations: CI = confidence interval; sRAGE = soluble receptor for advanced glycation end products.

for PGD associated with a 10-ng/ml increase in 6-hour sRAGE level was 1.23 (95% CI, 1.05–1.45; $P = 0.01$) and 24-hour level was 1.35 (95% CI, 1.01–1.81; $P = 0.044$). Adjustment for pulmonary artery systolic pressure or the use of cardiopulmonary bypass attenuated the relationship between both the 6-hour and 24-hour sRAGE levels and PGD.

Figure 2. Box and whisker plots of plasma receptor for advanced glycation end products (RAGE) levels at (A) 6 hours, and (B) 24 hours in cardiopulmonary bypass (CPB, $n = 109$) and patients with no cardiopulmonary bypass (No CPB, $n = 208$). Boxes display medians with interquartile ranges; error bars indicate 10th to 90th percentiles.

Association of sRAGE with Other Clinical Variables

Patients undergoing cardiopulmonary bypass had higher levels of sRAGE than those who did not (Figure 2). The median 6-hour sRAGE level was 10.5 ng/ml (IQR: 6.0, 20.6) in the 109 subjects with use of cardiopulmonary bypass versus 7.4 ng/ml (IQR: 3.6, 13.2) in the 208 subjects without ($P = 0.003$), and at 24 hours was 3.9 ng/ml (IQR: 2.1, 7.3) versus 1.9 ng/ml (IQR: 1.0, 3.8) in those without ($P < 0.001$). The volume of transfused red blood cells was associated with sRAGE at both 6 hours (Spearman $\rho = 0.21$, $P < 0.001$), and 24 hours ($\rho = 0.22$, $P < 0.001$). In multivariable linear regression models, the association of 6-hour sRAGE levels remained significant when simultaneously adjusting for transplant type, preoperative diagnosis, and use of cardiopulmonary bypass ($P = 0.020$); however, 24-hour sRAGE levels lost statistical significance on adjustment for the same variables ($P = 0.19$). The association of 6-hour sRAGE levels was strongest among subjects with PGD: at 6 hours both in unadjusted analysis ($\rho = 0.36$, $P = 0.002$), and adjusted analyses ($P < 0.001$ in a multivariable linear regression model simultaneously adjusting for transplant type, preoperative diagnosis, and use of cardiopulmonary bypass). In contrast, there was no correlation between pulmonary artery systolic pressure at the induction of anesthesia and sRAGE levels (Spearman's $\rho = 0.0005$; $P = 0.99$ at 6 h).

Higher sRAGE levels at 6 and 24 hours were associated with greater length of mechanical ventilation (Spearman $\rho = 0.34$, $P < 0.001$ at 6 h; $\rho = 0.38$, $P < 0.001$ at 24 h). However, sRAGE levels were not associated with total hospital length of stay at either time point (Spearman $\rho = 0.03$, $P = 0.69$ at 6 h; $\rho = 0.05$, $P = 0.50$ at 24 h). A total of 17 of 317 subjects died at 30 days, of whom 13 subjects (76%) had PGD. sRAGE levels were significantly higher in those who died: median sRAGE measured at 6 hours was 25.9 ng/ml (IQR: 5.1, 44.2) in those who died versus 7.7 ng/ml (IQR: 3.8, 14.1) in 30-day survivors ($P = 0.05$); and at 24 hours was 13.0 ng/ml (IQR: 4.9, 36.6) versus 2.3 ng/ml (IQR 1.3, 4.3) in 30-day survivors ($P < 0.001$).

DISCUSSION

PGD increases morbidity and mortality and affects longer-term outcomes after lung transplantation (1, 2, 8, 19). In this study, we demonstrated that elevated plasma levels of sRAGE in the post-transplant period are associated with an increased risk of PGD. In addition, sRAGE levels were higher in those subjects who underwent cardiopulmonary bypass and who received blood product transfusions.

In the lung, sRAGE appears primarily to be a marker of alveolar epithelial cell injury (16). Uchida and colleagues showed that sRAGE was present in high concentrations in the alveolar compartment in experimental and human lung injury (16), and sRAGE levels were associated with clinical outcomes in a large clinical trial of two mechanical ventilation strategies in patients with acute lung injury (25). In isolated perfused human lungs, airspace sRAGE levels were inversely correlated with the rate of alveolar fluid clearance, supporting the theory that sRAGE is a relevant marker of alveolar epithelial injury (26). Likewise, a clinical study of alveolar epithelial fluid transport function in patients with PGD suggests that the alveolar epithelium plays a major role in both the pathogenesis and resolution of PGD (27). Therefore, elevated plasma sRAGE levels in our PGD subjects may indicate alveolar epithelial injury.

RAGE may also produce vascular inflammation by perpetuating endothelial cell dysfunction, as RAGE and its ligands have been implicated in vascular atherogenesis through a variety of mechanisms (28–30). Indeed, endothelial RAGE serves as a counter-receptor for leukocyte integrins such as Mac-1 (31),

and the importance of leukocytes in the pathogenesis of acute lung injury had been well established. Because we did not perform RAGE expression analyses in cells from our subjects, we cannot specify the origin of the elevated plasma sRAGE levels in PGD (12).

Regardless of the cellular etiology, RAGE activation may be important in PGD. There is emerging evidence that RAGE blockade attenuates ischemia reperfusion injury in a murine pulmonary PGD model (32). Similar findings of the effect of RAGE blockade have been observed in hepatic ischemia reperfusion models (33, 34). Furthermore, administration of sRAGE attenuated lung injury in a murine model of LPS-induced lung injury suggesting a role for RAGE ligands in the augmentation of lung injury that is blocked by sRAGE (35). These data suggest a role for RAGE ligands in the augmentation of lung injury and should provide an impetus for further research into the relationship between RAGE, RAGE ligands, and the initiation and perpetuation of transplant-associated lung injury.

An important finding of our study was the association of plasma sRAGE levels with blood product transfusion. Accumulating evidence suggests an association between blood transfusion and the development of acute lung injury in the critically ill (36–38). The association of elevated sRAGE levels with red blood cell (RBC) transfusion may reflect increased alveolar or endothelial cell injury in patients receiving RBC transfusion, a finding that is supported by the stronger association between plasma sRAGE and RBC transfusion in patients with PGD in our study. Alternatively, RBC transfusion may promote cellular injury through activation of pathways initiated by the RAGE receptor. Recent evidence suggests that AGEs form in stored RBC units with increasing duration of storage (40). It is plausible that administration of RAGE ligands, such as RBC units containing AGEs, may increase detectable sRAGE levels in recipients, because RAGE expression is reported to be elevated in the presence of RAGE ligands (41). Further studies are necessary to determine the role of RBC transfusion and RAGE in the pathogenesis of PGD.

The association of sRAGE with PGD was in part attenuated by the use of cardiopulmonary bypass, and higher sRAGE levels were found in subjects who underwent cardiopulmonary bypass. The association of sRAGE levels with use of cardiopulmonary bypass could reflect alveolar epithelial injury that is caused by cardiopulmonary bypass itself. Alternately, the elevation of sRAGE levels associated with use of cardiopulmonary bypass could be due to systemic endothelial production of sRAGE (39). Similarly, the attenuation of the sRAGE association with PGD by elevated pulmonary artery pressures may indicate either shared pathophysiology, systemic inflammation, or simply may be an epiphenomenon of lung injury. In this cohort study, it was difficult to assess the individual contributions of elevated pulmonary artery pressures perhaps related to pretransplant diagnosis, use of bypass, and blood product transfusion, due to collinearity of these factors.

Our study is the first to show that plasma levels of sRAGE are elevated in clinical PGD. A prior study by Calfee and colleagues studied sRAGE at a single 4-hour time point in 20 subjects after lung transplantation, 7 of whom had grade 3 PGD (17). Our data are consistent with those of Calfee and colleagues in that RAGE levels were associated with length of mechanical ventilation. However, in contrast to our investigation, the prior study did not have sufficient power to test the association of RAGE with PGD.

Our study has several potential limitations. First, we did not measure preoperative RAGE levels in this study. Therefore, our ability to comment on the predictive value of RAGE is limited. Further, we measured RAGE levels in plasma, not

bronchoalveolar lavage fluid, and thus our ability to localize RAGE to alveolar epithelial cells may be limited (14). Likewise, we did not measure other concurrent markers to discern if the source of sRAGE was predominantly epithelial or endothelial. In addition, the clinical definition of PGD may not represent pathological acute lung injury in all cases, particularly in the early postoperative period. Other mimicking conditions may have caused some misclassification of this outcome.

In this multicenter study, we have shown that elevated sRAGE levels in the plasma are associated with PGD after lung transplantation. We also have demonstrated an association between sRAGE levels and use of cardiopulmonary bypass and administration of blood products. These results suggest the need for future basic and clinical studies into the role of RAGE in PGD and in the pathogenesis of acute lung injury. Further, our results suggest that investigation of RAGE blockade may lead to a novel therapeutic direction in PGD research.

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Participants in the Lung Transplant Outcomes Group by site: Columbia University: David Lederer, M.D., M.S. (P.I.), Selim Arcasoy, M.D., Joshua Sonett, M.D., Jessie Wilt, M.D., Frank D'Ovidio, M.D., Nilani Ravichandran, N.P., Matthew Bacchetta, M.D., Nadine Al-Naamani, M.D., Debbie Rybak, B.A., Michael Koeckert, B.A., Robert Sorabella, B.A. University of Pennsylvania (Coordinating site): Jason Christie, M.D., M.S. (P.I.), Steven M. Kawut, M.D., M.S., Alberto Pochettino, M.D., Y. Joseph Woo, M.D., Ejigayehu Demissie, M.S.N., Robert M. Kotloff, M.D., Vivek N. Ayha, M.D., James Lee, M.D., M.S., Denis Hadjiladis, M.D., M.H.S., Melanie Doran, B.S., Richard Aplenc, M.D., Clifford Deutschman, M.D., M.S., Benjamin Kohl, M.D. Vanderbilt University: Lorraine Ware, M.D. (P.I.), Stacy Kelley-Blackburn, R.N. Stanford University: Ann Weinacker, M.D., (P.I.), Ramona Doyle, M.D., Susan Spencer Jacobs, M.S.N., Val Scott, M.S.N. University of Alabama, Birmingham: Keith Wille, M.D. (P.I.), Joao de Andrade, M.D., Tonja Meadows, R.N. Johns Hopkins University: Jonathan Orens, M.D. (P.I.), Pali Shah, M.D., Ashish Shah, M.D., John McDyer, M.D. University of Michigan: Vibha Lama, M.D., M.S. (P.I.), Fernando Martinez, M.D., M.S., Emily Galopin, B.S. Duke University: Scott M. Palmer, M.D., M.H.S. (P.I.), David Zaas, M.D., M.B.A., R. Duane Davis, M.D., Ashley Finlen-Copeland, M.S.W.

References

- Christie JD, Bavaria JE, Palevsky HI, Litzky L, Blumenthal N, Kaiser LR, Kotloff RM. Primary graft failure following lung transplantation. *Chest* 1998;114:51–60.
- King RC, Binns OA, Rodriguez F, Kanithanon RC, Daniel TM, Spotnitz WD, Tribble CG, Kron IL. Reperfusion injury significantly impacts clinical outcome after pulmonary transplantation. *Ann Thorac Surg* 2000;69:1681–1685.
- Christie JD, Kotloff RM, Pochettino A, Arcasoy SM, Rosengard BR, Landis JR, Kimmel SE. Clinical risk factors for primary graft failure following lung transplantation. *Chest* 2003;124:1232–1241.
- Christie JD, Van Raemdonck D, de Perrot M, Barr M, Keshavjee S, Arcasoy S, Orens J. Report of the ISHLT Working Group on primary lung graft dysfunction part I: introduction and methods. *J Heart Lung Transplant* 2005;24:1451–1453.
- Christie JD, Robinson N, Ware LB, Plotnick M, De Andrade J, Lama V, Milstone A, Orens J, Weinacker A, Demissie E, 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.
- Arcasoy SM, Kotloff RM. Lung transplantation. *N Engl J Med* 1999;340:1081–1091.
- De Perrot M, Sekine Y, Fischer S, Waddell TK, McRae K, Liu M, Wigle DA, Keshavjee S. Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. *Am J Respir Crit Care Med* 2002;165:211–215.
- Christie JD, Sager JS, Kimmel SE, Ahya VN, Gaughan C, Blumenthal NP, Kotloff RM. Impact of primary graft failure on outcomes following lung transplantation. *Chest* 2005;127:161–165.
- Arcasoy SM, Fisher A, Hachem RR, Scavuzzo M, Ware LB. Report of the ISHLT Working Group on primary lung graft dysfunction part V: predictors and outcomes. *J Heart Lung Transplant* 2005;24:1483–1488.
- Christie JD, Kotloff RM, Ahya VN, Tino G, Pochettino A, Gaughan C, DeMissie E, Kimmel SE. The effect of primary graft dysfunction on survival after lung transplantation. *Am J Respir Crit Care Med* 2005;171:1312–1316.
- Krenn K, Klepetko W, Taghavi S, Lang G, Schneider B, Aharinejad S. Recipient vascular endothelial growth factor serum levels predict primary lung graft dysfunction. *Am J Transplant* 2007;7:700–706.
- de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion-induced lung injury. *Am J Respir Crit Care Med* 2003;167:490–511.
- Covarrubias M, Ware LB, Kawut SM, De Andrade J, Milstone A, Weinacker A, Orens J, Lama V, Wille K, Bellamy S, 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.
- Bierhaus A, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B, Stern DM, Nawroth PP. Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med* 2005;83:876–886.
- Fehrenbach H, Kasper M, Tschernig T, Shearman MS, Schuh D, Muller M. Receptor for advanced glycation endproducts (RAGE) exhibits highly differential cellular and subcellular localisation in rat and human lung. *Cell Mol Biol* 1998;44:1147–1157.
- Uchida T, Shirasawa M, Ware LB, Kojima K, Hata Y, Makita K, Mednick G, Matthay ZA, Matthay MA. Receptor for advanced glycation end-products is a marker of type I cell injury in acute lung injury. *Am J Respir Crit Care Med* 2006;173:1008–1015.
- Calfee CS, Budev MM, Matthay MA, Church G, Brady S, Uchida T, Ishizaka A, Lara A, Ranes JL, deCamp MM, et al. Plasma receptor for advanced glycation end-products predicts duration of ICU stay and mechanical ventilation in patients after lung transplantation. *J Heart Lung Transplant* 2007;26:675–680.
- Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D. 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.
- Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA, Patterson GA, Trulock EP, Hachem RR. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 2007;175:507–513.
- Oto T, Griffiths AP, Levvey BJ, Pilcher DV, Williams TJ, Snell GI. Definitions of primary graft dysfunction after lung transplantation: differences between bilateral and single lung transplantation. *J Thorac Cardiovasc Surg* 2006;132:140–147.
- Oto T, Levvey BJ, Snell GI. Potential refinements of the International Society for Heart and Lung Transplantation primary graft dysfunction grading system. *J Heart Lung Transplant* 2007;26:431–436.
- Whitson BA, Nath DS, Johnson AC, Walker AR, Prekker ME, Radosevich DM, Herrington CS, Dahlberg PS. Risk factors for primary graft dysfunction after lung transplantation. *J Thorac Cardiovasc Surg* 2006;131:73–80.
- Whitson BA, Prekker ME, Herrington CS, Whelan TP, Radosevich DM, Hertz MI, Dahlberg PS. Primary graft dysfunction and long-term

- pulmonary function after lung transplantation. *J Heart Lung Transplant* 2007;26:1004–1011.
24. Moldanado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol* 1993;138:923–936.
  25. Calfee CS, Ware LB, Eisner MD, Parsons PE, Thompson BT, Wickersham N, Matthay MA, Network NA. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 2008;63:1083–1089.
  26. Briot R, Frank JA, Uchida T, Lee JW, Calfee CS, Matthay MA. Elevated levels of RAGE, a marker of alveolar epithelial type I cell injury, predict impaired alveolar fluid clearance in isolated perfused human lungs. *Chest* 2009;135:269–275.
  27. Ware LB, Golden JA, Finkbeiner WE, Matthay MA. Alveolar epithelial fluid transport capacity in reperfusion lung injury after lung transplantation. *Am J Respir Crit Care Med* 1999;159:980–988.
  28. Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. *Circulation* 2002;105:816–822.
  29. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res* 1999;84:489–497.
  30. Wautier JL, Zoukourian C, Chappey O, Wautier MP, Guillausseau PJ, Cao R, Hori O, Stern D, Schmidt AM. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *J Clin Invest* 1996;97:238–243.
  31. Chavakis T, Bierhaus A, Al-Fakhri N, Schneider D, Witte S, Linn T, Nagashima M, Morser J, Arnold B, Preissner KT, *et al.* The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. *J Exp Med* 2003;198:1507–1515.
  32. Sternberg DI, Gowda R, Mehra D, Qu W, Weinberg A, Twaddell W, Sarkar J, Wallace A, Hudson B, D'Ovidio F, *et al.* Blockade of receptor for advanced glycation end product attenuates pulmonary reperfusion injury in mice. *J Thorac Cardiovasc Surg* 2008;136:1576–1585.
  33. Andrassy M, Volz HC, Igwe JC, Funke B, Eichberger SN, Kaya Z, Buss S, Autschbach F, Pleger ST, Lukic IK, *et al.* High-mobility group box-1 in ischemia-reperfusion injury of the heart. *Circulation* 2008;117:3216–3226.
  34. Zeng S, Feirt N, Goldstein M, Guarrera J, Ippagunta N, Ekong U, Dun H, Lu Y, Qu W, Schmidt AM, *et al.* Blockade of receptor for advanced glycation end product (RAGE) attenuates ischemia and reperfusion injury to the liver in mice. *Hepatology* 2004;39:422–432.
  35. Zhang H, Tasaka S, Shiraishi Y, Fukunaga K, Yamada W, Seki H, Ogawa Y, Miyamoto K, Nakano Y, Hasegawa N, *et al.* Role of soluble receptor for advanced glycation end-products on endotoxin-induced lung injury. *Am J Respir Crit Care Med* 2008;178:356–362.
  36. Gong MN, Thompson BT, Williams P, Pothier L, Boyce PD, Christiani DC. Clinical predictors of and mortality in acute respiratory distress syndrome: potential role of red cell transfusion. *Crit Care Med* 2005;33:1191–1198.
  37. Netzer G, Shah CV, Iwashyna TJ, Lanken PN, Finkel B, Fuchs B, Guo W, Christie JD. Association of RBC transfusion with mortality in patients with acute lung injury. *Chest* 2007;132:1116–1123.
  38. Zilberberg MD, Carter C, Lefebvre P, Raut M, Vekeman F, Duh MS, Shorr AF. Red blood cell transfusions and the risk of acute respiratory distress syndrome among the critically ill: a cohort study. *Crit Care* 2007;11:R63.
  39. Wautier JL, Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 2004;95:233–238.
  40. Lysenko L, Mierzchala M, Gamian A, Durek G, Kubler A, Kozlowski R, Sliwinski M. The effect of packed red blood cell storage on arachidonic acid and advanced glycation end-product formation. *Arch Immunol Ther Exp (Warsz)* 2006;54:357–362.
  41. Chavakis T, Bierhaus A, Nawroth PP. RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. *Microbes Infect* 2004;6:1219–1225.