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Plasma Intercellular Adhesion Molecule-1 and von Willebrand Factor in Primary Graft Dysfunction After Lung Transplantation

M. Covarrubias[a], L. B. Ware[a], S. M. Kawut[b,c], J. De Andrade[d], A. Milstone[a], A. Weinacker[e], J. Orens[f], V. Lama[g], K. Wille[d], S. Bellamy[h], C. Shah[h,i], E. Demissie[h] and J. D. Christie[h,i,*] for the Lung Transplant Outcomes Group

[a] *Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, Vanderbilt University, Nashville, TN*
[b] *Division of Allergy, Pulmonary and Critical Care Medicine, Columbia University, College of Physicians and Surgeons, New York, NY*
[c] *Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY*
[d] *Division of Pulmonary and Critical Care Medicine, University of Alabama, Birmingham, AL*
[e] *Division of Pulmonary and Critical Care Medicine, Stanford University, Stanford, CA*
[f] *Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University Hospital, Baltimore, MD*
[g] *Division of Allergy, Pulmonary and Critical Care Medicine, University of Michigan, Ann Arbor, MI*
[h] *Department of Biostatistics and Epidemiology and*
[i] *Division of Allergy, Pulmonary and Critical Care Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA*
* *Corresponding author: Jason D. Christie, jchristi@cceb.med.upenn.edu*

Primary graft dysfunction (PGD), a form of acute lung injury occurring within 72 h following lung transplantation, is characterized by pulmonary edema and diffuse alveolar damage. We hypothesized that higher concentrations of intercellular adhesion molecule-1 (ICAM-1) and von Willebrand factor (vWF) would be associated with the occurrence of PGD. A total of 128 lung transplant recipients among 7 lung transplant centers were enrolled in a multicenter, prospective, cohort study. Blood specimens were collected preoperatively and at 6, 24, 48 and 72 h following lung transplantation. The primary outcome was Grade 3 PGD at 72 h after transplant. Logistic regression and generalized estimating equations (GEE) were used to analyze plasma ICAM-1 and vWF. At each postoperative timepoint, mean plasma ICAM-1 concentrations were higher for patients with PGD versus no PGD. The GEE contrast estimate for the association of plasma ICAM-1 with PGD was 107.5 ng/mL (95% CI 38.7, 176.3), p = 0.002. In the multivariate analyses, this finding was independent of all clinical variables except pulmonary artery pressures prior to transplant. There was no association between plasma vWF levels and PGD. We conclude that higher levels of plasma ICAM-1 are associated with PGD following lung transplantation.

Key words: Epidemiology, human, injury mechanisms and biomarkers, ischemia/reperfusion injury, lung transplantation, translational research

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Introduction

Primary graft dysfunction (PGD) is a form of acute lung injury (ALI) occurring within 72 h following lung transplantation. Characterized by noncardiogenic pulmonary edema and diffuse alveolar damage, PGD occurs in 10–25% of lung transplant recipients and dramatically reduces short-term survival (1–4). A diagnosis of pulmonary arterial hypertension and high preoperative pulmonary artery pressures both increase the risk of PGD; however, the exact causes of PGD are unknown (5–8).

ALI is characterized by increased circulating plasma indicators of inflammation and endothelial dysfunction (9,10). ICAM-1 is a low molecular weight adhesion molecule that is highly expressed by Type-I pneumocytes, alveolar capillary endothelium and neutrophils. In patients with ALI, higher levels of plasma ICAM-1 were found to be associated with increased mortality and prolonged mechanical ventilation (9,10). The von Willebrand Factor is a large multimeric glycoprotein synthesized by endothelial cells and megakaryocytes. The vWF stabilizes Factor VIII and initiates the platelet plug. Endothelial injury leads to elevated vWF levels, whereas platelet-derived vWF does not significantly contribute to circulating levels. Elevated plasma levels of vWF are associated with worse outcomes in patients with pulmonary arterial hypertension, as well as patients with ALI (11–16).

We aimed to determine the association of postoperative levels and dynamic changes of ICAM-1 and vWF with PGD after lung transplantation. We hypothesized that higher

plasma levels of ICAM-1 and vWF would be associated with the occurrence of PGD following lung transplantation.

Materials and Methods

We performed a multicenter, prospective cohort study at seven lung transplant centers in the United States. The Lung Transplant Outcomes Group (LTOG) included the University of Pennsylvania, Vanderbilt University, University of Alabama at Birmingham, University of Michigan, Johns Hopkins University, Stanford University and Columbia University. One hundred and twenty-eight lung transplant recipients were enrolled between June 2003 and November 2004. Patients undergoing multi-organ transplantation were excluded. Details of the study sample have been previously published (17).

The primary outcome was Grade 3 PGD (hereafter referred to as PGD), defined as diffuse alveolar infiltrates in the lung allograft(s) on chest radiographs with a PaO_2/FiO_2 less than 200 mmHg, graded at 72 h. Further details of this definition have been previously published (2). Blood samples were collected preoperatively (at the time of admission for transplantation), and 6, 24, 48 and 72 hours following lung transplantation. Samples were processed within 30 min and stored at −80°C until analysis. Soluble ICAM-1 was measured in plasma by ELISA (R & D Systems, Inc., Minneapolis, MN) and expressed as ng/mL. The vWF in plasma was measured by ELISA (Diagnostica Stago, Parsippany, NJ) and expressed as a percentage of a normal pooled plasma control reference that has been assayed against a secondary standard of the Fourth International Standard of vWF. All samples were measured in duplicate. The coefficients of variation for ICAM-1 and vWF were both 3%. To place the findings of our cohort study in context, we also measured ICAM-1 levels in 10 normal, healthy controls, recruited from Vanderbilt University. Plasma vWF levels have previously been reported by our group (15).

Levels of ICAM-1 and vWF for patients with PGD were compared to patients without PGD using the Student's *t*-test or rank-sum tests as appropriate. Spearman rank correlations were used to compare continuous measures. Comparison of biomarkers by group variables (such as diagnosis category) was performed by ANOVA and ANCOVA. Dynamic biomarker profiles were plotted by PGD status and compared using generalized estimating equations (GEE), which are statistical models used to compare differences in longitudinal continuous measures.

Multivariable logistic regression included plasma biomarkers (independent variables), potential confounding variables, and PGD status (dependent variable). Variables with a p-value <0.20 on bivariate analysis with PGD status were considered potential confounders; such variables were included in the model one at a time to avoid overfitting. In addition, we used logistic regression to determine the association between biomarker level and mortality at 30 days after transplantation. Statistical analyses were performed using STATA version 9.2 (STATA data Corporation, College Station, TX) and SAS version 9.1 (SAS Institute, Cary, NC).

Informed consent was obtained for enrollment prior to lung transplantation, and the research protocol was approved by the Institutional Review Board of each center.

Results

Baseline characteristics of the subjects enrolled in the cohort study are shown in Table 1 and have been previously reported in part (17). Six subjects died within 30 days of transplant. Twenty-six patients (20%, 95% CI 14%, 28%) met the criteria for PGD. Characteristics associated with a higher risk of PGD included: recipient diagnosis of diffuse parenchymal lung disease or pulmonary arterial hypertension, bilateral lung transplantation, use of cardiopulmonary bypass and high pulmonary artery systolic pressure at induction of anesthesia.

Table 1: Donor and recipient characteristics

Donor variables	No PGD n = 102	PGD n = 26	p-Value
Age (years)	30 (28,33)	32 (25,39)	0.54
Female gender	37%	43%	0.59
Race/ethnicity			0.28
Caucasian	66%	76%	
African American	21%	5%	
Hispanic	13%	14%	
Other	1%	5%	
Recipient and surgical variables			
Age (years)	52 (49,55)	45 (39,53)	0.06
Female gender	47%	31%	0.11
Race/ethnicity			0.06
Caucasian	90%	81%	
African American	7%	12%	
Hispanic	2%	0%	
Other	1%	8%	
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
Use of inhaled nitric oxide	9%	20%	0.18
Pulmonary artery systolic pressure at induction (mmHg)	40 (37,43)	61 (45,77)	<0.001

Adapted in part from reference 17. Continuous variables are expressed as means with 95% confidence intervals and dichotomous variables as column percentages.

Plasma ICAM-1 levels were higher in PGD patients than in others at every postoperative time point ($p < 0.05$ at 6 h, $p < 0.0001$ at 24 h and $p < 0.001$ at 48 and 72 h) (Figure 1). Preoperative plasma levels of ICAM-1 also were higher in patients who went on to develop PGD, but this difference did not reach statistical significance ($p = 0.059$). The contrast estimate derived from GEE was 107.5 ng/mL ($p = 0.002$ and 95% CI 39, 176) indicating that, on average, the ICAM-1 level was over 100 ng/mL higher in PGD patients compared to non-PGD patients over the 72-h postoperative time course.

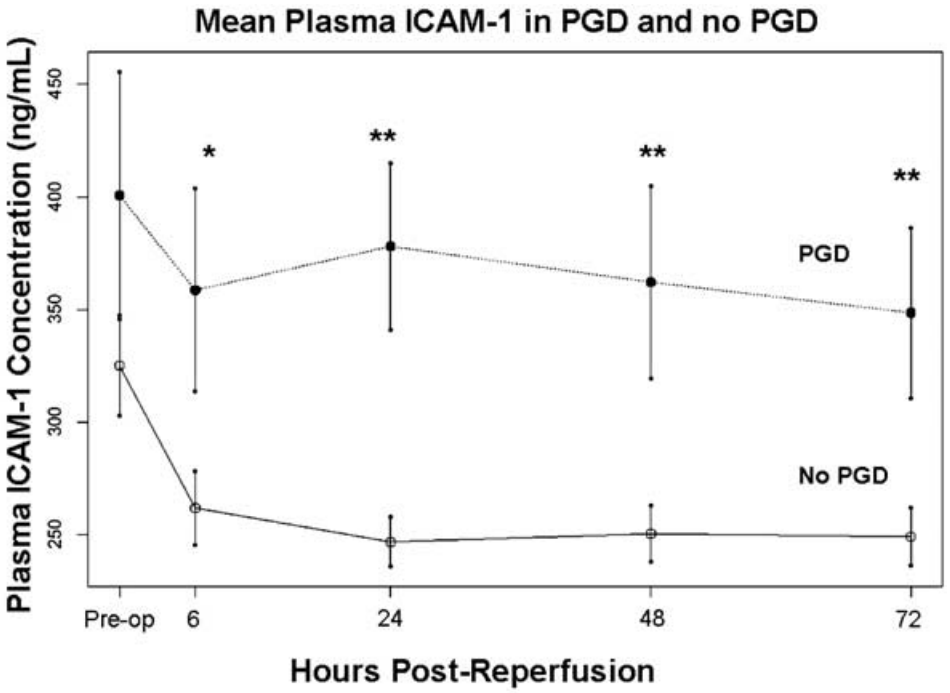

Figure 1: Mean pre- and postoperative plasma intercellular adhesion molecule-1 (ICAM-1) concentrations over time for lung transplant recipients with and without primary graft dysfunction (PGD). Data are presented as mean ± standard error. *$p < 0.05$, **$p < 0.001$ for PGD versus no PGD.

We then tested the association of 24-h ICAM-1 levels with the risk of PGD after adjustment for potential confounders (Table 2). The increase in concentrations of ICAM-1 was independent of clinical variables such as ischemic time, transfusion, diagnosis category or use of cardiopulmonary bypass or nitric oxide. However, inclusion of pulmonary artery systolic pressure in the model attenuated the relationship between ICAM-1 levels and PGD. Perhaps explaining this attenuation, there was a correlation between pulmonary artery systolic pressure at induction and ICAM-1 levels measured preoperatively (Spearman's rho = 0.36, $p = 0.0039$) and at 24 h post-reperfusion (Spearman's rho = 0.23, $p = 0.036$). Furthermore, preoperative plasma ICAM-1 levels differed according to diagnosis category (ANOVA r-square = 0.41, $p < 0.001$). The lowest preoperative ICAM-1 levels were among those with COPD (mean +/− standard deviation: 250.7 +/− 93.8), higher among CF (457.8 +/− 132.8) and IPF (429.1 +/− 194.7) and highest in congenital heart disease (647.8 +/− 33.1). These differences in preoperative ICAM-1 level were independent of pulmonary artery systolic pressure measured at induction (ANCOVA r-square = 0.50, $p < 0.001$). In general, preoperative levels in these conditions were higher than in the 10 normal controls measured (mean 311.1 +/− 36.6).

Higher plasma levels of ICAM-1 were associated with an increase in 30-day mortality (Figure 2). The mean +/− standard deviation ICAM-1 level at 24 h among those who died was 500 +/− 187 versus 262 +/− 107 in those who survived ($p < 0.0001$). Among patients with PGD, higher 24-h ICAM-1 levels appeared to confer an increased risk of 30-day mortality, but this was inconclusive: among 26 PGD patients, the mean ICAM-1 level was 469 +/− 191 in PGD subjects who died (n = 5) versus 343 +/− 156 in PGD subjects who survived (n = 21), $p = 0.15$.

Patients with PGD had preoperative plasma vWF levels similar to those of patients without PGD (Figure 3). No association between recipient clinical variables and preoperative vWF levels was observed. Plasma levels of vWF increased in a linear fashion beginning 6 h postoperatively

Table 2: Bivariate and multivariable analyses of the association of ICAM-1 levels at 24 h with PGD

	Odds ratio for PGD[1] (95% CI)	p-Value
Unadjusted base model for ICAM-1 level	2.12 (1.39,3.23)	0.001
ICAM-1 level adjusted for:		
Recipient age	2.06 (1.33, 3.18)	0.001
Recipient gender	2.93 (1.56, 5.51)	0.001
Recipient diagnosis	1.89 (1.20, 2.98)	0.006
Recipient race/ethnicity	2.14 (1.40, 3.28)	<0.001
Procedure type	2.00 (1.30, 3.10)	0.002
Cardiopulmonary bypass	1.76 (1.13, 2.75)	0.013
Use of nitric oxide	2.07 (1.35, 3.15)	0.001
Total ischemic time	2.10 (1.37, 3.23)	0.001
Pulmonary artery systolic pressure at transplant	1.54 (0.91, 2.60)	0.109
Blood mL during first 24 h	1.92 (1.24, 3.00)	0.004
Platelet mL during first 24 h	1.94 (1.23, 3.06)	0.005
Fresh frozen plasma mL during first 24 h	1.86 (1.16, 2.99)	0.010

[1]Per 100 ng/mL increase in ICAM-1 level.

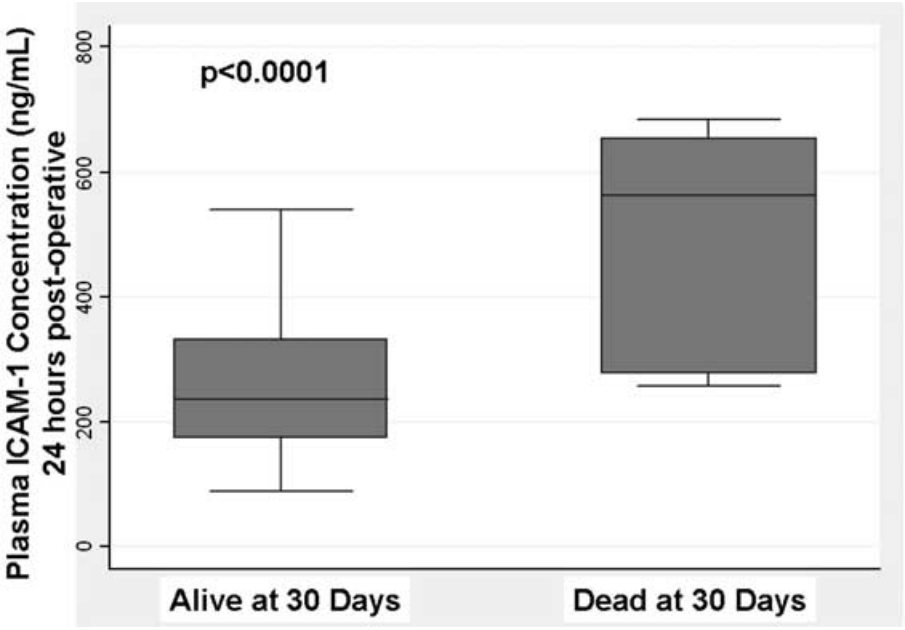

Figure 2: Plasma intercellular adhesion molecule-1 (ICAM-1) measured at 24 h and 30-day mortality. Medians and interquartile ranges between subjects alive at 30 days and dead at 30 days are presented.

over the 72-h time period with separation of the curves beginning at 24 h; however, these findings did not approach statistical significance, except at 72 h ($p = 0.054$). There were no associations of plasma vWF levels at any time point with mortality.

Discussion

PGD results in significant morbidity and is the leading cause of death in the first 30 days after lung transplantation (1–4). We sought to determine whether plasma markers that reflect inflammation and epithelial and endothelial injury in the transplant recipient would be associated with the development of PGD. We found that higher plasma levels of ICAM-1 at all time points within the first 72 h after lung transplantation were associated with PGD. In contrast, increased plasma levels of vWF early after transplant were not associated with PGD, although vWF levels did tend to be higher in patients with PGD at 72 h after transplantation.

The expression of ICAM-1 in PGD following lung transplantation has been described in animal models (18,19). During

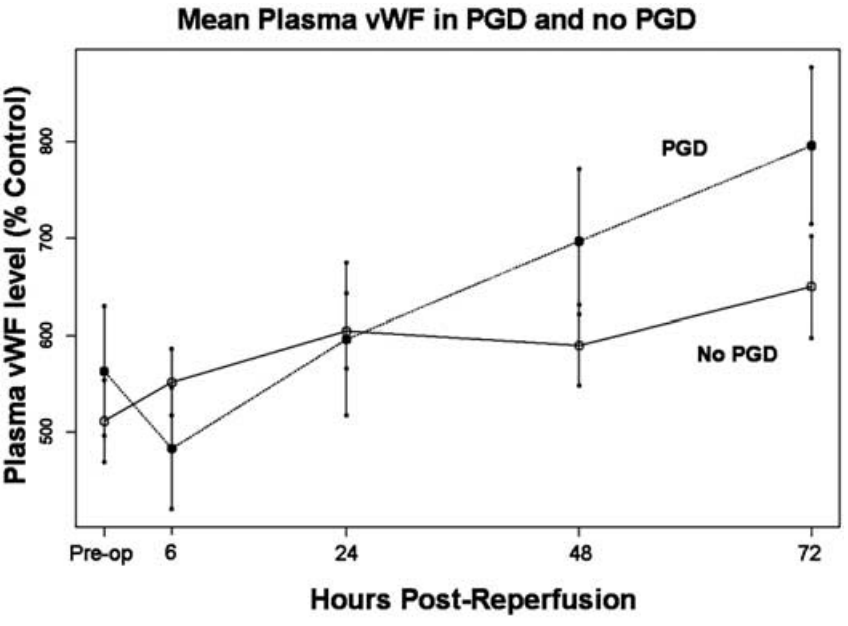

Figure 3: Mean pre- and postoperative plasma von Willebrand factor antigen (vWF) concentrations over time for lung transplant recipients with and without primary graft dysfunction (PGD). Data are presented as mean ± standard error.

pulmonary reperfusion in rats transplanted with lungs donated after cardiac death, an increase in ICAM-1 expression was observed in alveolar tissue (18,19). This increased expression was attenuated by administration of ICAM-1 anti-sense oligonucleotides instilled into the pulmonary vasculature during the preservation period. Suppression of ICAM-1 expression led to significant improvements in physiological outcomes including oxygenation, pulmonary vascular resistance, pulmonary blood flow and recipient survival (18,19). Therefore, in addition to being a potential biomarker of PGD, ICAM-1 might also contribute to the pathogenesis of PGD and could represent a novel therapeutic target.

We found an association between plasma ICAM-1 levels and pulmonary artery pressure before transplantation. This association has also been described in sickle cell patients with pulmonary hypertension (20). This observation is thought to be a consequence of release of ICAM-1 from endothelial cells due to leukocyte activation and deficits in nitric oxide production. Nitric oxide production normally suppresses ICAM-1 expression. ICAM-1 could, therefore, provide a mechanistic link between preoperative pulmonary hypertension and PGD and serve to prime circulating macrophages, which infiltrate the lung allograft(s). Furthermore, preoperative plasma ICAM-1 levels were lowest among subjects with a diagnosis of COPD. This finding was independent of the degree of pulmonary hypertension. Therefore, upregulation of ICAM-1 may also reflect the preoperative inflammatory states associated with cystic fibrosis and idiopathic pneumonia syndromes, independent of pulmonary hypertension. This notion is further supported by the fact that plasma ICAM-1 levels were higher in these conditions than normal controls. Alternatively, higher ICAM-1 concentrations may simply be an epiphenomenon of pulmonary hypertension, preoperative diagnosis and acute lung injury.

Despite extremely high levels in the entire transplant cohort, plasma levels of vWF did not differ between the two groups except at 72 h ($p = 0.054$). This finding is surprising considering that the plasma levels of vWF in this study are substantially higher than plasma levels in patients enrolled in a large clinical trial of patients with ALI and acute respiratory distress syndrome (ARDS) (16) and are approximately 10-fold higher than levels reported in normal controls (15). Furthermore, the lack of association of plasma vWF levels with PGD contrasts with other forms of ALI and ARDS where elevated plasma levels of vWF early in the course of ALI and ARDS are predictive of death, prolonged mechanical ventilation and organ failure (14–16). These findings suggest that markers of endothelial damage may not be the best predictors of graft function in the early postoperative period of lung transplantation, considering the vWF levels appeared so high across all transplant subjects. In contrast, measures of epithelial integrity may be more revealing in early PGD. Interestingly, in a small study of patients with severe PGD, the edema fluid-to-plasma protein ratio, a measure of alveolar-capillary barrier permeability, was also not associated with patient outcomes whereas the rate of alveolar fluid clearance, a marker of alveolar epithelial integrity was (21).

There are some limitations to this study. First, we chose Grade 3 PGD as the primary outcome, which represents the most severe form of the disease. However, recently published studies demonstrate that Grade 3 PGD is most predictive of morbidity and mortality in lung transplant recipients (22). The levels of ICAM-1 and vWF measured in our study in other grades of PGD (Grades 0–2) were not significantly different (data not shown), demonstrating that Grade 3 PGD is clearly distinct. Second, although our study had adequate power to detect differences in biomarkers between PGD groups, we lacked sufficient power to completely explore effects on mortality since only 6 subjects died and, therefore, multivariable models were not possible. Future studies aimed at prediction of mortality will need to include more patients. Third, we measured ICAM-1 preoperatively at the time of transplantation. Thus the utility of preoperative ICAM-1 measurement for prediction of PGD will need to be assessed with consideration for the hours required for the test to be performed. Similar issues apply to the recent finding that preoperative VEGF levels are associated with PGD (23). Future studies may need to focus on earlier measurement to provide adequate time to impact management. Fourth, as this is a human observational study, we are uncertain if this link represents a causal one, or simply an epiphenomenon. The elevation of ICAM-1 levels may simply reflect the treatments and systemic illness that go along with severe PGD. Further laboratory and human research will be needed both to establish causality as well as to assess clinical utility of ICAM measurement in practice.

In a prior publication in the same cohort of subjects, we found a similar relationship between type-1 plasminogen activator inhibitor (PAI-1) and both pulmonary hypertension and PGD (17). In the current study, we have used this same resource to expand our focus to include measurement of ICAM-1 and vWF. Thus the generalizability of our findings to other populations may be limited.

In summary, we have shown that plasma levels of ICAM-1 are associated with PGD in human lung transplant recipients. Furthermore, preoperative ICAM-1 levels correlate with pulmonary hypertension and diagnosis category. These findings, taken together with animal studies, suggest that ICAM-1 might play a pathogenic role in clinical PGD. Studies targeting ICAM-1 activity and/or production in recipients or during organ preservation may be useful future directions toward preventing PGD.

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