Title
Cytokines and clinical predictors in distinguishing pulmonary transfusion reactions.

Journal
Transfusion, 55(8)

ISSN
0041-1132

Authors
Roubinian, Nareg H
Looney, Mark R
Kor, Daryl J
et al.

Publication Date
2015-08-01

DOI
10.1111/trf.13021

Peer reviewed

Cytokines and clinical predictors in distinguishing pulmonary transfusion reactions

Nareg H. Roubinian,[1,2] Mark R. Looney,[2] Daryl J. Kor,[3] Clifford A. Lowell,[1] Ognjen Gajic,[3] Rolf D. Hubmayr,[3] Michael A. Gropper,[2] Monique Koenigsberg,[2] Gregory A. Wilson,[3] Michael A. Matthay,[2] Pearl Toy,[2] Edward L. Murphy,[1,2] and the TRALI Study Group

BACKGROUND: Pulmonary transfusion reactions are important complications of blood transfusion, yet differentiating these clinical syndromes is diagnostically challenging. We hypothesized that biologic markers of inflammation could be used in conjunction with clinical predictors to distinguish transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and possible TRALI.

STUDY DESIGN AND METHODS: In a nested case-control study performed at the University of California at San Francisco and Mayo Clinic, Rochester, we evaluated clinical data and blood samples drawn before and after transfusion in patients with TRALI (n = 70), possible TRALI (n = 48), TACO (n = 29), and controls (n = 147). Cytokines measured included granulocyte-macrophage–colony-stimulating factor, interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-α. Logistic regression and receiver operating characteristics curve analyses were used to determine the accuracy of clinical predictors and laboratory markers in differentiating TACO, TRALI, and possible TRALI.

RESULTS: Before and after transfusion, IL-6 and IL-8 were elevated in patients with TRALI and possible TRALI relative to controls, and IL-10 was elevated in patients with TACO and possible TRALI relative to that of TRALI and controls. For all pulmonary transfusion reactions, the combination of clinical variables and cytokine measurements displayed optimal diagnostic performance, and the model comparing TACO and TRALI correctly classified 92% of cases relative to expert panel diagnoses.

CONCLUSIONS: Before transfusion, there is evidence of systemic inflammation in patients who develop TRALI and possible TRALI but not TACO. A predictive model incorporating readily available clinical and cytokine data effectively differentiated transfusion-related respiratory complications such as TRALI and TACO.

Pulmonary complications of transfusion include transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and perhaps other types of lung injury such as possible TRALI. Traditionally, pulmonary edema in these syndromes have been divided into hydrostatic (TACO) and nonhydrostatic or inflammatory etiologies (TRALI and possible TRALI) with the distinction of the latter two diagnoses being the temporal relationship to an alternative risk factor for acute respiratory distress syndrome, such as sepsis or pneumonia.[1]

Distinguishing the precise etiology of posttransfusion pulmonary edema often poses a diagnostic challenge in critically ill patients. These determinations are important with distinct differences in management and prognosis of these specific clinical entities.[2] Biomarkers have

ABBREVIATIONS: ALI = acute lung injury; AUC = area under the curve; BNP(s) = brain natriuretic peptide(s); HLGOF = Hosmer-Lemeshow goodness of fit; ROC = receiver operating characteristic; TACO = transfusion-associated circulatory overload.

From the [1]Blood Systems Research Institute and the [2]University of California at San Francisco, San Francisco, California; and the [3]Mayo Clinic, Rochester, Minnesota.

Address reprint requests to: Nareg H. Roubinian, MD, MPHTM, Blood Systems Research Institute 270 Masonic Avenue, San Francisco CA 94118; e-mail: nroubinian@bloodsystems.org

This project was supported by NHLBI SCCOR Grant P50-HL-1027 (PT) and NHLBI Career Grant K24-HL-7135 (ELM). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NHLBI or the NIH.

Received for publication October 14, 2014; revision received December 22, 2014; and accepted January 1, 2015.

doi:10.1111/trf.13021

TRANSFUSION 2015;00;00–00

increasingly been utilized in clinical diagnosis and decision making. For example, the use of biomarkers such as troponin and brain natriuretic peptide (BNP) has transformed the identification and management of patients with myocardial infarction and pulmonary edema, respectively.[3-6]

The role of biomarkers in differentiating the etiology of transfusion-related lung edema is less clear, with at least one study noting limited diagnostic value in the use of BNPs.[7] More recently, plasma biomarkers representing inflammatory pathways have been found to predict the onset and underlying pathogenesis of nonhydrostatic (e.g., permeability) pulmonary edema.[8,9] Pretransfusion aberrations in these inflammatory cytokines have also been recognized in patients who develop TRALI.[10,11] However, the role of inflammatory cytokines in facilitating the discrimination of TRALI and TACO has not been reported. We hypothesized that plasma-derived markers of inflammation could be used in conjunction with relevant clinical predictors to improve the classification of pulmonary transfusion reactions.

MATERIALS AND METHODS

The protocol was approved, including a waiver of consent, by the University of California San Francisco Medical Center and Mayo Clinic institutional review boards. The study was funded by the National Heart, Lung and Blood Institute, which played no role in its design, execution, or analysis.

Study design and subjects

This investigation utilized a nested case-control study design. Study participants were identified from a previously conducted prospective cohort investigation. Details of this study population have been previously described.[10] Briefly, prospective surveillance for pulmonary transfusion reactions was conducted between 2006 and 2009 at the University of California San Francisco Medical Center and the Mayo Clinic (Rochester, MN). Cases of pulmonary transfusion reactions were identified by active surveillance using a real-time electronic method that screened arterial blood gas results in patients older than 6 months who received blood transfusion, as previously described.[12]

Electronic alerts were generated for any transfused patient with hypoxemia on arterial blood gas analysis ($PaO_2/FiO_2 < 300$ mmHg) within 12 hours of blood product issue from the blood bank. Nurse coordinators with critical care training identified cases of possible transfusion reactions based on acute posttransfusion hypoxemia and findings of bilateral opacities on the chest radiograph, triggering the collection of relevant clinical data via chart review. Cases were then reviewed by two critical care physicians on a four-member expert panel. Each expert independently classified a case as TRALI, TACO, possible TRALI, TACO and TRALI when one could not distinguish between the two diagnoses, or as "other" when an alternative diagnosis was identified.

TRALI was defined as new acute lung injury (ALI) that developed during or within 6 hours of transfusion, and there was no temporal relationship to an alternative risk factor for ALI. Possible TRALI was defined as new ALI that developed during or within 6 hours of transfusion, and there was a clear temporal relationship to an alternative risk factor for ALI. A diagnosis of TACO was derived from criteria used in the Centers for Disease Control Biovigilance System, with pulmonary edema developing within 6 hours of transfusion and being characterized by clinical, echocardiographic, or laboratory evidence of left atrial hypertension.[13]

Controls were selected using a stratified sampling scheme from among all transfused patients at the same hospital and during the same time period as enrollment of cases. Controls did not have evidence of hypoxemia during or within 12 hours after transfusion of the last unit nor evidence of bilateral infiltrates on chest radiography. They were sampled from strata based on number of blood units transfused (1 to 2, 3 to 9, and 10 or more) and study center.[10]

Clinical predictors

Characteristics in the limited clinical data set included age, sex, race, hospital location of transfusion (e.g., intensive care unit, operating room), number of units of any component transfused within 6 hours of developing pulmonary edema, a history of congestive heart failure, acute renal failure, chronic renal failure, hemodialysis, recent surgery, and type of surgery before developing edema (Appendix S1, available as supporting information in the online version of this paper). Additional variables in the full clinical model included APACHE II score, number of units of any component transfused within 24 hours of developing pulmonary edema, and receipt of mechanical ventilation before or after blood transfusion. Hemodynamic variables included heart rate, systolic and diastolic blood pressures, and vasopressor administration before or after blood transfusion. Central venous pressures, echocardiographic variables, BNP values, peak airway pressures, and PaO_2/FiO_2 ratios were compared in the subset of patients for whom these variables were available.

Patient sample collection and cytokine assays

Residual routine pre- and posttransfusion recipient blood samples were collected from the clinical laboratory for both cases and controls and stored at 4°C before processing. Plasma fractions were separated from whole blood EDTA tubes (purple top) and stored at −80°C before

measurement of cytokines. Samples tested were those collected closest to transfusion.

Plasma cytokines were measured using microarray kits from Bio-Rad (Hercules, CA) on the Luminex platform (Luminex Corporation, Austin, TX; Appendix S2, available as supporting information in the online version of this paper). Cytokines measured in cases of TACO, TRALI, possible TRALI, and controls included granulocyte-macrophage–colony-stimulating factor (GM-CSF), interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α).

Statistical analysis

Distributions and proportions of demographic and clinical data were tabulated for groups of pulmonary transfusion reactions. Data were expressed as mean values ± standard deviation (SD), median, or proportions and were compared among groups using analysis of variance and Kruskal-Wallis test, if appropriate. Cytokine data were imported into a statistical analysis package (Stata Version 12.1, StataCorp, College Station, TX). Undetectable values were assigned a value of zero for the purposes of analysis. Because the distribution of cytokine results was skewed, the Wilcoxon test was used to compare cytokine levels in samples between groups of TRALI, TACO, possible TRALI, and control patients, and results were log-transformed for regression analyses. A two-tailed p value of less than 0.05 was considered significant.

Logistic regression was performed to determine individual associations between possible risk factors. A multivariable model of risk factors was developed by including variables significant in the initial analysis ($p < 0.20$). Clinical predictors (echocardiographic and invasive hemodynamic data) available in a limited subset of patients (<80%) were not included in models. Receiver operating characteristic (ROC) curves were constructed and area under the curve (AUC) was calculated to determine the discriminative capacity of the clinical predictors and laboratory markers in differentiating TACO, TRALI, and possible TRALI relative to expert panel diagnoses. Confidence intervals (95% CIs) for the AUC were obtained using a bootstrap method with 1000 repetitions. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit (HLGOF) test. Sensitivity, specificity, positive and negative likelihood ratios, and correct classification were calculated for the clinical and cytokine models independently as well as the combined clinical and cytokine models. These metrics were compared for clinical predictors in the full cohort and the subset of patients where cytokine data were available.

RESULTS

Electronic surveillance generated 14,308 hypoxemia alerts on 11,488 patients out of a total of 47,783 patients transfused 463,207 blood components during the study period. Study nurses excluded 13,747 alerts for a variety of reasons, including no evidence of pulmonary edema, stable existing pulmonary edema, no chest radiograph performed, and recent lung transplant.

The expert panel reviewed 561 alerts with new or worsening bilateral pulmonary opacities and diagnosed 166 cases of TACO, 94 cases of TRALI, 153 cases of ALI or possible TRALI, 47 cases of TACO and TRALI, and 101 cases of miscellaneous causes of bilateral pulmonary opacities such as atelectasis or pleural effusions.[10] A total of 163 controls were selected from 36,335 patients who received blood products from May 2006 through August 2009 and did not have hypoxemia. Complete clinical information was available for 89 cases of TRALI, 145 cases of possible TRALI, and 83 cases of TACO (Appendix S3, available as supporting information in the online version of this paper). Five of the 94 cases of TRALI were related to bacterial contamination of a blood component or anaphylaxis and not included in the final data set. Of the 166 cases of TACO, relevant clinical information was available for the last 83 consecutive cases, from February 2008 through August 2009. Complete clinical data were available for 145 of the 153 cases of possible TRALI, and the most frequent ALI risk factors identified were sepsis (n = 68), shock (n = 59), pneumonia (n = 22), and aspiration (n = 16).

Tables 1 and 2 provide a description of the clinical characteristics and comorbid risk factors of cases of TACO, TRALI, and possible TRALI, respectively. As previously reported, patients with TACO were older and had a greater prevalence of cardiac and renal comorbidities as well as recent surgery, specifically cardiac surgery.[14] Patients with TRALI were more likely to have had recent spine or liver surgery.[10] Patients with possible TRALI were more likely to be located in the intensive care unit, received fewer transfused units, and had a greater prevalence of acute renal failure. APACHE II scores were similar among the three groups.

Table 3 provides ventilator and hemodynamic characteristics of patients with TACO, TRALI, and possible TRALI. TACO patients had lower systolic blood pressures, higher central venous pressures, and a greater prevalence of echocardiographic abnormalities, and were more likely to require vasopressor support before the onset of edema. TACO patients more commonly received mechanical ventilation and had higher PaO_2/FiO_2 ratios before the onset of pulmonary edema compared with TRALI and possible TRALI patients. Before transfusion, BNP levels were significantly higher in patients with TACO compared to TRALI and possible TRALI, and these differences in TACO and TRALI patients persisted after the development of pulmonary edema.

Plasma cytokines

Clinical samples were only available for a subset of transfusion recipients and were captured for possible TRALI

TABLE 1. Cohort characteristics*

Patient characteristics	TACO (n = 83)	TRALI (n = 89)	Possible TRALI (n = 145)	p value
Age (years)	61 ± 20	54 ± 20	58 ± 19	<0.01
Sex				
Female	49 (59.0)	45 (50.6)	70 (48.3)	0.29
Race				
White	61 (73.5)	63 (70.8)	122 (74.8)	0.62
Nonwhite	5 (6.0)	10 (11.2)	19 (11.7)	
Missing/not reported	17 (20.5)	16 (18.0)	22 (13.5)	
Transfusions (number of units)				
1 or 2	31 (37.3)	27 (30.3)	67 (46.2)	<0.01
3-9	30 (36.1)	33 (37.1)	58 (40.0)	
10+	22 (26.5)	29 (32.6)	20 (13.8)	
Patient location				
Ward	9 (10.8)	18 (20.2)	27 (18.6)	0.04
Intensive care unit	22 (26.5)	27 (30.3)	79 (54.5)	
Operating room	52 (62.7)	43 (48.3)	39 (26.9)	
Outpatient	0	1 (1.1)	0	
Study location				
Mayo Clinic	63 (75.9)	45 (50.6)	98 (67.6)	<0.01
University of California San Francisco Medical Center	20 (24.1)	44 (49.4)	47 (32.4)	

* Data are reported as mean ± SD or number (%).

TABLE 2. Comorbid risk factors*

Risk factor	TACO (n = 83)	TRALI (n = 89)	Possible TRALI (n = 145)	p value
History of congestive heart failure	29 (35)	7 (8)	8 (5)	<0.01
Coronary artery disease	34 (41)	15 (17)	15 (22)	<0.01
Atrial fibrillation	20 (24)	8 (9)	16 (11)	<0.01
Acute renal failure	15 (18)	23 (26)	36 (25)	0.36
Chronic renal failure	15 (18)	5 (6)	15 (10)	0.31
Hemodialysis	14 (17)	10(11)	18 (12)	0.51
Severe liver disease	9 (11)	21 (24)	27 (19)	0.09
Recent surgery	62 (75)	54 (61)	55 (38)	<0.01
Cardiac surgery	41 (49)	12 (13)	7 (5)	<0.01
Liver surgery	4 (5)	15 (17)	2 (1)	<0.01
Spine surgery	1 (1)	11 (12)	1 (1)	<0.01

* Data are reported as number (%).

and TACO patients from February 2008 through August 2009. Cytokine analysis was performed in pre- and posttransfusion samples of 70 patients with TRALI, 48 patients with possible TRALI, 29 patients with TACO, and 147 control patients. The median times that blood was drawn before and after the onset of edema were 21 (interquartile range [IQR], 9-55) and 16 (IQR, 3-48) hours, respectively.

Comparison of pre- and posttransfusion cytokine values in TRALI, TACO, possible TRALI, and control patients is summarized in Table 4. IL-6, IL-8, IL-10, and TNF-α were significantly different ($p < 0.05$) between cases and controls in univariate analysis. Pretransfusion plasma IL-6 and IL-8 levels were elevated in TRALI and possible TRALI patients relative to controls but not TACO patients relative to controls. Pretransfusion TNF-α levels were higher in TRALI and possible TRALI patients relative to TACO patients. Posttransfusion IL-6 but not IL-8 levels were elevated in TACO patients relative to controls. In both pre- and post-transfusion samples, IL-10 levels were higher in TACO and possible TRALI patients relative to TRALI patients and controls. In both pre- and posttransfusion samples, IL-8 levels were significantly higher in possible TRALI patients compared to those of patients with TACO.

Regression models

Clinical predictors (see Materials and Methods and Appendix S1) and cytokine levels were used to construct diagnostic models for TRALI versus TACO (Table 5), TACO versus possible TRALI, and TRALI versus possible TRALI. Models using clinical predictors had excellent and similar discrimination for TACO versus TRALI (AUC, 0.88; 95% CI, 0.81-0.95; HLGOF $p = 0.58$), TACO versus possible TRALI (AUC, 0.89; 95% CI, 0.85-0.94; HLGOF $p = 0.17$), and possible TRALI versus TRALI (AUC, 0.86; 95% CI, 0.81-0.91; HLGOF $p = 0.30$) (Table 5 and Appendix S4, available as

TABLE 3. Clinical characteristics*

Characteristic	TACO (n = 83)	TRALI (n = 89)	Possible TRALI (n = 145)	p value
Ventilation before edema	56 (77)	42 (47)	76 (58)	<0.01
Peak airway pressure (mmHg; n = 246)	29	30	33	0.03
PaO_2/FiO_2				
Before edema (n = 148)	320	329	294	0.23
After edema (n = 278)	177	156	152	0.15
Mean HR before edema	86	88	100	<0.01
% with HR > 100				
Before edema	23%	23%	49%	<0.01
After edema	26%	45%	57%	<0.01
% with SBP < 100 before edema	44%	29%	30%	0.05
Vasopressors				
Before edema	46 (55)	35 (39)	59 (41)	0.05
After edema	45 (54)	39 (44)	86 (59)	0.07
APACHE II scores	13 ± 7	14 ± 7	14 ± 6	0.09
Echo				
WMA (n = 159)	13 (16)	5 (6)	12 (8)	0.06
Valve abnormality (n = 157)	16 (19)	8 (9)	2 (1)	<0.01
Ejection fraction % (n = 157)	58 ± 16	65 ± 9	62 ± 11	0.02
Fluid balance before edema (L)	3.7 (1.6-6)	3.7 (1.6-7.3)	4.4 (1.5-7.2)	0.59
CVP				
Before edema (n = 154)	12	9	13	<0.01
After edema (n = 202)	13	10	11	<0.01
BNP				
Before edema (n = 72)†‡	1004	70	298	<0.01
After edema (n = 67)‡§	1907	252	693	<0.01

* Data are reported as number (%), mean ± SD, or median (IQR).
† Median BNP levels between possible TRALI and TACO significantly different.
‡ Median BNP levels between TACO and TRALI significantly different.
§ Median BNP levels between possible TRALI and TRALI significantly different.
‖ CVP = central venous pressure; HR = heart rate; SBP = systolic blood pressure; WMA = wall motion abnormality.

supporting information in the online version of this paper). The AUC for clinical models of the full cohort and the subset which had cytokines were similar (Appendix S5, available as supporting information in the online version of this paper).

Similarly, the model using five pretransfusion cytokines (IL-6, IL-8, IL-10, GM-CSF, and TNF-α) had excellent discrimination for the diagnosis of TRALI versus TACO (AUC, 0.88; 95% CI, 0.80-0.95; HLGOF p = 0.79; Table 5). By contrast, the AUC for diagnosis using single cytokines was poor to fair ranging from 0.51 for IL-6 to 0.77 for IL-8. Using the five pretransfusion cytokines (IL-6, IL-8, IL-10, GM-CSF, and TNF-α), the AUC was 0.78 (95% CI, 0.67-0.89; HLGOF p = 0.36) for the diagnosis of possible TRALI versus TACO and 0.80 (95% CI, 0.72-0.88; HLGOF p = 0.53) for the diagnosis of TRALI versus possible TRALI (Appendix S4). Discrimination of transfusion reactions using posttransfusion cytokines was lower for all comparisons (TRALI vs. TACO—AUC, 0.81; 95% CI, 0.71-0.91; HLGOF p = 0.09; TACO vs. possible TRALI —AUC, 0.76; 95% CI, 65-0.88; HLGOF p = 0.25; and TRALI vs. possible TRALI—AUC, 0.74; 95% CI, 0.65-0.83; HLGOF p = 0.50).

The combined clinical and cytokine models had improved discrimination for TACO versus TRALI (AUC, 0.96; 95% CI, 0.92-1.0; HLGOF p = 0.36), TACO versus possible TRALI (AUC, 0.93; 95% CI, 0.87-0.99; HLGOF p = 0.16), and possible TRALI versus TRALI (AUC, 0.89; 95% CI, 0.83-0.95; HLGOF p = 0.60). Combined models had similar discrimination and accuracy utilizing either pre- or posttransfusion cytokine levels. Models including limited clinical and cytokine data had similar discrimination and accuracy relative to models with more extensive clinical data (Table 5).

DISCUSSION

The diagnoses of pulmonary transfusion reactions are based on clinical definitions that lack both sensitivity and specificity.[15] Distinguishing these clinical entities requires the interpretation of clinical, radiographic, and hemodynamic data that are labor-intensive to extract.[16] The standard in research studies of these reactions with disparate pathophysiology has been to utilize a panel of expert clinicians with expertise in both intensive care and transfusion medicine.[7,10,17] The goal of this study was to examine the performance of relevant clinical predictors as well as a panel of candidate cytokines, individually and when combined together in the discrimination of TACO, TRALI, and possible TRALI relative to expert panel review.

We have confirmed that TRALI and possible TRALI are preceded by elevated plasma levels of IL-6 and IL-8 relative to controls.[11,18] These findings of inflammation may represent the first insult in the two-event hypothesis

TABLE 4. Pre- and posttransfusion cytokine levels* (pg/mL)

Transfusion category	IL-6		IL-8		IL-10		TNF-α		GM-CSF	
	Pretransfusion‡	Posttransfusion†	Pretransfusion‡	Posttransfusion‡	Pretransfusion§	Posttransfusion§	Pretransfusion‡§	Posttransfusion	Pretransfusion	Posttransfusion
TRALI (n = 70)	37‖ (13-87)	100‖ (27-262)	35‖ (13-85)	35‖ (14-120)	8 (3-26)	14 (7-54)	15 (6-42)	13 (5-56)	14 (6-35)	17 (4-62)
TACO (n = 29)	28 (13-87)	112‖ (47-350)	28 (14-47)	28 (12-64)	27‖ (18-88)	41‖ (19-82)	7 (3-14)	9 (4-21)	25 (8-38)	25 (9-59)
Possible TRALI (n = 48)	86‖ (24-577)	232‖ (62-1129)	42‖ (18-188)	65‖ (21-357)	51‖ (17-136)	57‖ (29-112)	15 (6-42)	18 (6-41)	23 (7-91)	30(8-109)
Controls (n = 147)	24 (10-64)	47 (20-114)	25 (10-55)	18 (10-42)	9 (4-28)	11 (5-24)	12 (5-60)	13 (5-56)	23 (7-95)	21 (5-87)

* Data are reported as median (IQR).
† Cytokine levels between possible TRALI and TRALI significantly different.
‡ Cytokine levels between possible TRALI and TACO significantly different.
§ Cytokine levels between TACO and TRALI significantly different.
‖ Cytokine levels significantly different relative to matched controls.

that has been postulated to explain the occurrence of nonhydrostatic pulmonary edema in critically ill patients.[19,20] The transfusion of a blood product containing either antibodies or factors that accumulate during storage then provides a second insult, signaling for neutrophil-mediated endothelial damage and lung injury. Conversely, TACO was not associated with systemic elevations in IL-6 and IL-8 before transfusion. These findings support the clinical criteria that this syndrome is one of hydrostatic rather than inflammatory pulmonary edema.

Acute and chronic inflammatory conditions have been associated with elevations in anti-inflammatory cytokines.[21,22] In this study, elevated systemic levels of IL-10 were seen before transfusion in TACO and possible TRALI compared to controls and TRALI patients. Elevations in IL-10 have been described in cardiac and renal conditions often associated with TACO.[23-25] A possible explanation for this differential elevation of proinflammatory and anti-inflammatory mediators in possible TRALI cases may relate to the temporal relationship to an alternative ALI risk factor in possible TRALI cases that preceded transfusion.[19] These cases had differential elevations in IL-6 and IL-8 levels relative to other cases and controls and have previously been associated with higher mortality rates relative to TRALI and TACO.[7,19]

Logistic regression models that utilized a panel of inflammatory cytokines had substantially superior performance to a single marker for differentiating patients with pulmonary transfusion reactions as evaluated by ROC curve analysis. The best performing cytokine for the diagnosis of TRALI (IL-8) was similar to that identified in previous studies.[10,11] However, the best-performing cytokine for differentiating pulmonary transfusion reactions from one another was the anti-inflammatory cytokine IL-10. Pretransfusion cytokines provided better discrimination of reactions in comparison to posttransfusion markers.[26] This finding may to some extent relate to the high prevalence of surgical transfusion events in this cohort with postoperative inflammatory changes mitigating differences in each group. For example, the increase in IL-6 in posttransfusion TACO patients may be due to tissue injury after cardiac or vascular surgery rather than transfusion.

Individually, our independent models including either clinical or cytokine predictors resulted in modest discrimination of pulmonary transfusion reactions. This level of discrimination raises concern for misclassification when applied to individualized decisions for care delivery. Furthermore, improved diagnostic accuracy is desired for transfusion reactions that require reporting to external agencies such as with TRALI and TACO. In contrast, our data suggest that models incorporating limited clinical data with inflammatory cytokines provide excellent diagnostic accuracy certainly for TACO and TRALI with correct classification consistently exceeding 80% relative to that

TABLE 5. Performance characteristics of regression models

Regression models	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Correctly classified (%)
TACO vs. TRALI (n = 99)*						
Clinical model	0.88	90	66	86	73	83
Cytokine model	0.88	93	59	84	77	83
Combined model	0.96	93	90	96	84	92
Limited clinical/cytokine						
TACO vs. TRALI (n = 99)*	0.97	96	83	93	88	92
TACO vs. possible TRALI (n = 77)*	0.93	91	89	93	86	91
TRALI vs. possible TRALI (n = 118)*	0.87	83	72	81	74	78

* Numbers in parentheses represent the combined numbers of subjects compared in the model.
NPV = negative predictive value; PPV = positive predictive value.

of an expert panel consensus diagnosis. While many of the clinical predictors required intensive chart review and granular capture of hemodynamic physiology, the strongest predictors of outcome were clinical comorbidities available to most clinicians. A model combining cytokine levels and key clinical variables may ultimately be the most efficient way to provide an accurate diagnosis of syndromes such as TACO and TRALI.

This study has both strengths and limitations. Major strengths include the use of active surveillance in a study population composed of both medical and surgical patients, the detailed collection of granular clinical data, and expert panel review to ensure accuracy of the outcome adjudication. However, several limitations should also be noted. Our study did not collect extensive clinical information on all cases of TACO, and plasma samples were only available on a subset of the full cohort. Each of these factors limited our ability to detect between-group differences and precluded development of a validation cohort. Notably, the sensitivity analyses of clinical predictors in the full cohort as well as those with clinical samples showed qualitatively similar results. An additional limitation is the small subset of cytokines evaluated. Certainly, this was not an exhaustive examination of all potential biomarkers that might be useful when attempting to differentiate the various pulmonary transfusion reactions. Nevertheless, our evaluation of the specific cytokines studied in this investigation provides important information regarding their relative value when attempting to determine the etiology of respiratory compromise after transfusion.

Challenges associated with case adjudication may also have led to misclassification of the respiratory complication by the expert panel. To the extent that this may have occurred, it would certainly have impacted our study findings. Moreover, it is increasingly recognized that both forms of pulmonary edema (hydrostatic and nonhydrostatic) may coexist.[27] The overlapping nature of pulmonary edema may further confound the ability of our predictive models to accurately differentiate the transfusion-related respiratory complications investigated. Finally, case adjudication was at times limited by the unavailability of clinical data. To address these concerns, we intentionally excluded cases where the expert panel could not distinguish these two entities with some degree of certainty (TACO and TRALI cases). In addition to validating the findings of this cohort, future studies could examine inflammatory cytokines and cardiopulmonary biomarkers, such as BNP, in the classification of these cases without diagnostic certainty or where adequate clinical data is not available.

Several studies have shown that pulmonary transfusion reactions are underreported.[10,28,29] Given the advent of electronic medical record surveillance and focus on adverse clinical outcomes related to transfusion, we can expect increased identification of complex cases of posttransfusion pulmonary edema.[30-32] While providing some guidance in their identification, definitions of pulmonary transfusion reactions require more specific criteria to help differentiate complicated clinical cases. Identifying key clinical and laboratory predictors and developing algorithms that incorporate the pathophysiology of these specific clinical entities may assist clinicians in appropriately managing donors and recipients alike.

In conclusion, elevation of inflammatory cytokines in TRALI supports the two-hit hypothesis. The elevation is also consistent with often inflammatory recipient risk factors in possible TRALI. Their lack of elevation in TACO supports the concept that it is a noninflammatory condition. Independently, clinical predictors and inflammatory plasma cytokines provided moderate clinical discrimination for the diagnosis of specific pulmonary transfusion reactions. However, the combination of clinical predictors and inflammatory cytokines improved the accuracy of prediction models allowing a high rate of appropriate classification of pulmonary transfusion reactions. If validated in future studies, the addition of inflammatory cytokines to clinical risk factors may prove useful when

attempting to determine the underlying etiology of post-transfusion pulmonary edema.

ACKNOWLEDGMENTS

Transfusion-Related Acute Lung Injury Study Group members are as follows:

Steering committee:

Pearl Toy, MD (Principal Investigator), Ognjen Gajic, MD, Mark Looney, MD, Rolf Hubmayr, MD, Michael A. Gropper, MD, PhD, Michael Matthay, MD, Richard B. Weiskopf, MD, Edward L. Murphy, MD, MPH, and Clifford Lowell, MD, PhD

Clinical site coordinators and research assistants:

University of California at San Francisco: Monique Koenigsberg, RN, Kelly Lang, RN, Christopher Chin, Deanna Lee, PhD, and Lynda Bartek, RN

Mayo Clinic: Gregory Wilson, CCRC, Tami Krpata, Deborah Rasmussen, and Cindy Medcalfe

Blood banks and investigators:

Blood Centers of the Pacific: Nora Hirschler, MD

Blood Systems Research Institute: Rosa Sanchez Rosen, MD, Philip Norris, MD, and Dan Hindes

University of California San Francisco: Pearl Toy, MD

Mayo Clinic: S. Breandan Moore, MD, Jeffrey L. Winters, MD, and Manish Gandhi, MD

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Popovsky MA. Pulmonary consequences of transfusion: TRALI and TACO. Transfus Apher Sci 2006; 34:243-4.
2. Li G, Kojicic M, Reriani MK, et al. Long-term survival and quality of life after transfusion associated pulmonary edema in critically ill medical patients. Chest 2010; 137:783-9.
3. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002; 347:161-7.
4. Mueller C, Scholer A, Laule-Kilian K, et al. Use of B-type natriuretic peptide in the evaluation and management of acute dyspnea. N Engl J Med 2004; 350:647-54.
5. Hamm CW, Goldmann BU, Heeschen C, et al. Emergency room triage of patients with acute chest pain by means of rapid testing for cardiac troponin T or troponin I. N Engl J Med 1997; 337:1648-53.
6. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med. 1996; 335:1342-9.
7. Li G, Daniels CE, Kojicic M, et al. The accuracy of natriuretic peptides (brain natriuretic peptide and N-terminal pro-brain natriuretic) in the differentiation between transfusion-related acute lung injury and transfusion-related circulatory overload in the critically ill. Transfusion 2009; 49:13-20.
8. Agrawal A, Zhuo H, Brady S, et al. Pathogenetic and predictive value of biomarkers in patients with ALI and lower severity of illness: results from two clinical trials. Am J Physiol Lung Cell Mol Physiol 2012; 303:L634-9.
9. Agrawal A, Matthay MA, Kangelaris KN, et al. Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients. Am J Respir Crit Care Med 2013; 187:736-42.
10. Toy P, Gajic O, Bacchetti P, et al.; TRALI Study Group. Transfusion-related acute lung injury: incidence and risk factors. Blood 2012; 119:1757-67.
11. Vlaar AP, Hofstra JJ, Determann RM, et al. Transfusion-related acute lung injury in cardiac surgery patients is characterized by pulmonary inflammation and coagulopathy: a prospective nested case-control study. Crit Care Med 2012; 40:2813-20.
12. Finlay HE, Cassorla L, Feiner J, Toy P. Designing and testing a computer-based screening system for transfusion-related acute lung injury. Am J Clin Pathol 2005; 124:601-9.
13. Division of Healthcare Quality Promotion, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC). The National Healthcare Safety Network (NHSN) Manual, Biovigilance Component. Atlanta, GA: CDC; Jul 2010.
14. Murphy EL, Kwaan N, Looney MR, et al.; TRALI Study Group. Risk factors and outcomes in transfusion-associated circulatory overload. Am J Med 2013; 126:357.e29-38.
15. Skeate RC, Eastlund T. Distinguishing between transfusion related acute lung injury and transfusion associated circulatory overload. Curr Opin Hematol 2007; 14:682-7.
16. Gajic O, Gropper MA, Hubmayr RD. Pulmonary edema after transfusion: how to differentiate transfusion-associated circulatory overload from transfusion-related acute lung injury. Crit Care Med 2006; 34:S109-13.
17. Kleinman SH, Triulzi DJ, Murphy EL, et al. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study-II. The Leukocyte Antibody Prevalence Study-II (LAPS-II): a retrospective cohort study of transfusion-related acute lung injury in recipients of high-plasma-volume human leukocyte antigen antibody-positive or -negative components. Transfusion 2011; 51:2078-91.
18. Looney MR, Roubinian N, Gajic O, et al.; Transfusion-Related Acute Lung Injury Study Group. Prospective study on the clinical course and outcomes in transfusion-related acute lung injury. Crit Care Med 2014; 42:1676-87.
19. Triulzi DJ. Transfusion-related acute lung injury: an update. Hematol Am Soc Hematol Educ Program 2006; 497-501.
20. Looney MR, Gropper MA, Matthay MA. Transfusion-related acute lung injury: a review. Chest 2004; 126:249-58.
21. Dennis KL, Blatner NR, Gounari F, Khazaie K. Current status of interleukin-10 and regulatory T-cells in cancer. Curr Opin Oncol 2013; 25:637-45.
22. Filippi CM, von Herrath MG. IL-10 and the resolution of infections. J Pathol 2008; 214:224-30.

23. Stenvinkel P, Ketteler M, Johnson RJ, et al. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia—the good, the bad, and the ugly. Kidney Int 2005; 67:1216-33.
24. O'Meara E, Rouleau JL, White M, et al.; ANCHOR Investigators. Heart failure with anemia: novel findings on the roles of renal disease, interleukins, and specific left ventricular remodeling processes. Circ Heart Fail 2014; 7:773-81.
25. Aukrust P, Gullestad L, Uel T, et al. Inflammatory and anti-inflammatory cytokines in chronic heart failure: potential therapeutic implications. Ann Med 2005; 37:74-85.
26. Jawa RS, Anillo S, Huntoon K, et al. Interleukin-6 in surgery, trauma, and critical care part II: clinical implications. J Intensive Care Med 2011; 26:73-87.
27. National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network, Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. N Engl J Med 2006; 354:2564-75.
28. Narick C, Triulzi DJ, Yazer MH. Transfusion-associated circulatory overload after plasma transfusion. Transfusion 2012; 52:160-5.
29. Clifford L, Singh A, Wilson GA, et al. Electronic health record surveillance algorithms facilitate the detection of transfusion-related pulmonary complications. Transfusion 2013; 53:1205-16.
30. Cohn CS, Welbig J, Bowman R, et al. A data-driven approach to patient blood management. Transfusion 2014; 54:316-22.
31. McWilliams B, Triulzi DJ, Waters JH, et al. Trends in RBC ordering and use after implementing adaptive alerts in the electronic computerized physician order entry system. Am J Clin Pathol 2014; 141:534-41.
32. Yazer MH, Triulzi DJ, Reddy V, Waters JH. Effectiveness of a real-time clinical decision support system for computerized physician order entry of plasma orders. Transfusion 2013; 53:3120-7.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's Web site:
Appendix S1. List of clinical predictors used in regression models.
Appendix S2. Blood sample processing and cytokine analysis.
Appendix S3. Cohort subtotals with clinical data and blood samples.
Appendix S4. Sensitivity analyses for full and cytokine subset cohorts.
Appendix S5. Regression analyses for clinical, cytokine, and combined models.