

Transfusion-related acute lung injury: incidence and risk factors

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Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related mortality. To determine TRALI incidence by prospective, active surveillance and to identify risk factors by a case-control study, 2 academic medical centers enrolled 89 cases and 164 transfused controls. Recipient risk factors identified by multivariate analysis were higher IL-8 levels, liver surgery, chronic alcohol abuse, shock, higher peak airway pressure while being mechanically ventilated,

current smoking, and positive fluid balance. Transfusion risk factors were receipt of plasma or whole blood from female donors (odds ratio = 4.5, 95% confidence interval [CI], 1.85-11.2, $P = .001$), volume of HLA class II antibody with normalized background ratio more than 27.5 (OR = 1.92/100 mL, 95% CI, 1.08-3.4, $P = .03$), and volume of anti-human neutrophil antigen positive by granulocyte immunofluorescence test (OR = 1.71/100 mL, 95% CI, 1.18-2.5, $P = .004$). Little

or no risk was associated with older red blood cell units, noncognate or weak cognate class II antibody, or class I antibody. Reduced transfusion of plasma from female donors was concurrent with reduced TRALI incidence: 2.57 (95% CI, 1.72-3.86) in 2006 versus 0.81 (95% CI, 0.44-1.49) in 2009 per 10 000 transfused units ($P = .002$). The identified risk factors provide potential targets for reducing residual TRALI. (*Blood*. 2012;119(7):1757-1767)

Introduction

Since 2003, the FDA has documented that the leading cause of transfusion-related fatality has been transfusion-related acute lung injury (TRALI),¹ defined as acute lung injury (ALI)² that develops during or within 6 hours after transfusion of one or more units of blood or blood components.^{3,4} Included in this definition are cases of ALI after multiple transfusions, a well-known ALI risk factor.⁵ The condition has been under-reported since the first description in 1985 by Popovsky and Moore,⁶ and the overall incidence has been reported only by passive surveillance studies. Although risk factors have been identified in subgroups, such as critically ill patients⁷ and cardiac surgery patients,⁸ risk factors in recipients and in transfused blood products (eg, antibody, bioreactive substances, older RBC storage age⁹) have not been identified in the general population of transfused patients because of the lack of a large prospective, case-controlled study. The goal of this study was to prospectively determine incidence by an active surveillance system¹⁰ implemented at 2 large academic centers. During the course of this study, the American Association of Blood Banks recommended the reduction of transfusion of plasma and platelets from donors potentially harboring alloantibodies,^{11,12} thus making it possible to measure any change in TRALI incidence that was concurrent with implementation of this recommendation. The other goal of this study was to determine transfusion and recipient risk factors by enrolling concurrent transfused controls without TRALI.

Methods

See supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Study design

Active surveillance of TRALI was conducted at 2 tertiary care medical centers: the University of California—San Francisco (UCSF), San Francisco, CA and the Mayo Clinic, Rochester, MN. Enrollment began on March 1, 2006; the case-control study ended on August 31, 2009 and surveillance ended on December 31, 2009. All RBC and platelet units transfused during the study period were leuko-reduced. All patients older than 6 months were prospectively evaluated in real time for hypoxemia ($\text{PaO}_2/\text{FiO}_2 < 300$ mmHg) within 12 hours after issue of any blood component from the blood bank, by continuous electronic surveillance of arterial blood gas (ABG) results, and blood bank records in the hospital laboratory information system (Oztech Systems).¹⁰ Given the 6-hour window for the acute onset of TRALI by definition, most cases would have an ABG result within 12 hours. Cases without FiO_2 in ABG reports would be missed. After receiving an electronic alert in real time, coordinators gathered and entered patient data into a Web-based database (QuesGen Systems) and sent an electronic summary, including history, laboratory data, chest radiographs, and radiologist reports of chest radiographs, to the expert panel for review, usually within 72 hours of the alert.

Reviewers were blinded to all information regarding transfused units, including component type. TRALI was diagnosed by concurrence of 2 expert physicians by predetermined criteria (Table 1). At least monthly, conference calls were conducted with site investigators and coordinators,

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Table 1. Expert panel criteria for adjudication of acute posttransfusion hypoxemia with bilateral pulmonary infiltrates

	TRALI	TACO	TACO/TRALI	Transfused ALI‡	Other
Bilateral infiltrates on chest radiograph consistent with acute pulmonary edema*	Yes	Yes	Yes	Yes	No
Clinical evidence of left atrial hypertension as the principal explanation for acute pulmonary edema†	No	Yes	Indeterminate§	No	NA
Presence of major ALI risk factor(s)‡	No	Yes/No	Yes/No	Yes	NA

NA indicates not applicable.

*Atelectasis, pleural effusions, chronic interstitial infiltrates, or uninterpretable chest x-rays (resulting from poor quality) were considered not to be consistent with acute pulmonary edema.

†A systematic integrated evaluation of hemodynamic monitoring (PAOP, CVP), echocardiography (ejection fraction, E/E'), chest radiographic imaging (vascular pedicle width, cardiothoracic ratio), laboratory findings (BNP and NT-ProBNP), and clinical course (rapid within hours resolution of acute respiratory failure after preload/afterload reduction would suggest hydrostatic TACO rather than ALI permeability pulmonary edema).

‡Major ALI risk factors included pneumonia, sepsis, aspiration, multiple fractures, and pancreatitis. "Transfused ALI" corresponds to "possible TRALI" according to the Canadian Consensus Conference.³ To select clear TRALI cases, these "possible TRALI" patients were not included in this study.

§Insufficient data to make accurate determination or both conditions coexist.

||Exception: If a patient with a major ALI risk factor was stable, the risk factor did not appear to cause ALI, and ALI was precipitated by the transfusion, then the patient was considered to have TRALI.

and disputable cases were reviewed by the full panel of 4 experts by conference call.

Implementation of plasma from male donors (Mayo), and plasma from male and never pregnant female donors (UCSF) occurred in 2007 to 2008, whereas reduction of platelets from previously pregnant females (UCSF) and predominantly male donors (Mayo) was partially implemented in 2008. Recipient tracing of components from donors associated with TRALI cases was not performed.

Because the study was observational and testing was performed on residual clinical laboratory specimens, cases and controls were enrolled without written informed consent. The protocol was approved by the institutional review board at each institution.

Definition of TRALI cases and controls

TRALI was defined as new ALI that developed during or within 6 hours of transfusion of one or more units, not attributable to another ALI risk factor.⁴ The study was designed to detect cases of TRALI that, by definition ($\text{PaO}_2/\text{FiO}_2 < 300$ mmHg), had severe enough hypoxemia that the patient's physician on any service would probably obtain ABG measurement(s) for patient management. The study was not designed to detect mild cases¹³ that do not meet the $\text{PaO}_2/\text{FiO}_2$ less than 300-mmHg criterion for ALI. To include patients with the highest likelihood of TRALI, we excluded patients who had another major ALI risk factor (pneumonia, sepsis, aspiration, multiple fractures, and pancreatitis), except if that patient was stable, the risk factor did not appear to cause ALI, and the ALI was precipitated by transfusion. "Possible TRALI"³ cases with another major ALI risk factor were designated "transfused ALI" and not included in the case-control study.

Control patients were randomly selected transfusion recipients who had no pulmonary signs or symptoms within 12 hours after transfusion of the last unit. We stratified sampling by number of units transfused, because this was probably a strong risk factor⁵ and having too few controls with large numbers of units transfused would make assessment of other risk factors more difficult. Controls were stratified according to the number of units transfused to the recipient (regardless of component type): low (1-2 units), medium (3-9 units), and high volume (≥ 10 units) transfusions.

Laboratory test methods

Case and control samples were performed in the same runs to reduce reagent lot variability effects.

Recipient HLA antigen typing

Low resolution class I HLA-A, B, Cw and class II HLA-DRB1, DRB3/4/5, DQB1 typing was performed using the LABType SSO (One Lambda).

Antibodies to HLA

Screening for antibody to HLA class I (anti-HLA-class I) and antibody to HLA class II (anti-HLA-class II) was performed using LABScreen Mixed

LSM12 (One Lambda). A normalized background (NBG) ratio more than 2.2 was considered a positive result and the positive samples were further tested for antibody specificity using the LABScreen Single-antigen Bead assay (One Lambda) to identify antibody specificity. A specific antibody was considered positive if the MFI was more than 300 mmHg and strong if the MFI was more than 2500 mmHg for class I, or more than 1500 mmHg for class II.¹⁴ HLA antibody strength was also assessed by the NBG ratio result of the screening test.¹⁵

Antibody to HNA

Screening for anti-human neutrophil antigen (HNA) was performed using the granulocyte agglutination test (GAT) and granulocyte immunofluorescence test (GIFT), analyzed by a flow cytometer (EPICS XL/MCL Flow Cytometer Beckman Coulter). GIFT was interpreted as positive if cells from at least 1 panel donor were interpreted as positive. Quantitative determination of antibody strength by the GIFT was assessed (see supplemental Methods).

Determining anti-HNA specificity

Anti-HNA specificity was determined by 2 methods: (1) by examining the reactivity pattern obtained from samples interpreted as positive in the GAT and/or GIFT (eg, for anti-HNA-3a); and (2) by the monoclonal antibody immobilization of neutrophil antigens assay, where monoclonal antibodies were used to capture glycoproteins expressing HNA-1a, HNA-1b, HNA-1c, HNA-2a, HNA-4a, and HNA-5a alloantigens.

Bioreactive substances

Lysophosphatidylcholine concentrations were determined by liquid chromatography-tandem mass spectrometry. Cytokine levels, including vascular endothelial growth factor in plasma of patients, and donor units were determined using Luminex multiplex bead assays as described by the manufacturer (Bio-Rad). The levels of soluble CD40 ligand and platelet factor 4 in plasma samples were determined using standard plate-bound ELISA format assays. Methods were used as described by the manufacturer (soluble CD40 ligand kit from Bender MedSystems; platelet factor 4 kit from R&D Systems).

To determine neutrophil priming activity, samples were examined on a dual-laser flow cytometer (FACScan Flow Cytometer, BD Biosciences) and analyzed using FlowJo Version 9.2 software (TreeStar). To detect plasma-induced CD11b/CD66 up-regulation, donor neutrophils were incubated with test plasma and then stained for CD11b/CD66b: (1) plasma from the neutrophil donor incubated with his/her own neutrophils, negative control; (2) donor neutrophils incubated with InM fMLF/BSA, positive control; (3) donor neutrophils incubated with plasma test sample, showing lack of reactivity; and (4) donor neutrophils incubated with plasma test sample, showing significant up-regulation of CD11b/CD66b. Numbers in each quadrant designate percentage of positive cells based on negative control.

Statistical methods

TRALI incidence analysis. Steps to reduce the incidence of TRALI (TRALI mitigation) were implemented from 2007 to 2008. In 2006, neither center had started mitigation. In 2009, both sites had completed mitigation. Incidences in 2006 and 2009 were compared. To assess trend, monthly counts of TRALI cases were modeled by Poisson regression, controlling for the number of transfusions in each month.

Risk factor analysis. TRALI occurs in patients rather than in units; thus, our analyses treated each patient as one observation. In keeping with the case-control design, the primary outcome was whether the patient was a TRALI case or a control. Regarding potential recipient risk factors, we analyzed measurements made in the recipient before, and not during, the transfusion period of 6 hours before pulmonary edema, to avoid measuring the effects of any TRALI prodrome.

We defined and evaluated a large number of potential risk factors. Recipient factors evaluated were potential or known risk factors for ALI. Transfusion factors evaluated were donor gender, donor pregnancy, component type, storage age of RBC, platelet and plasma units, patient and donor ABO compatibility, anti-HNA, anti-HLA, MICA antibody, lysoPC, leukocyte and platelet cytokines, and neutrophil priming activity. The quantity of a potential transfusion predictor (antibody or bioreactive substance) in a transfused unit was calculated by the predictor strength/concentration multiplied by the plasma volume estimated for each positive component. All candidate risk factors were evaluated by logistic regression. To permit estimation of the effect of number of involved units, the SAS SurveyLogistic procedure¹⁶ was used for initial analyses. Because initial results indicated that virtually all the risk of increasing number of transfused units could be explained by other variables in multivariate models, simpler stratified conditional logistic regression methods were subsequently used for the primary results. Multiple imputation methods¹⁷ were used to prevent deletion of patients when any one of their units had a missing measurement, to preserve more information and introduce less bias than alternative methods.

Analysis of storage age of RBC units. We used as predictors the numbers of RBC units received by each patient that were above certain unit age cutoffs. These can never be reduced by addition of fresher units, and they were well defined (zero) for those who did not receive any units above the specified unit age cutoff.

Multivariate model building. We hypothesized that the number of units transfused might be an important risk factor for TRALI. To prevent confounding with the number of units from producing spurious results for other risk factors, we controlled for the number of units received during or within 6 hours of TRALI (or the corresponding artificial TRALI time created for controls), which we called “involved units,” in all models used to initially evaluate potential risk factors. Thus, a patient characteristic associated with needing many units would not appear to be risky unless it also increased risk by some other mechanism. We also controlled for study site (UCSF or Mayo), as is usual practice for multisite studies. Multivariate models were built stepwise, selecting risk factors with small *P* values for addition and/or factors that no longer had small *P* values for deletion at each step, taking into account biologic plausibility.

Descriptive data. We do not provide statistical comparisons of descriptive summaries (Tables 2 to 6) because (1) they do not take account of the stratified sampling, and (2) they would reverse the correct roles of dependent and independent variables. TRALI versus control is the dependent variable in risk factor analyses.

Results

During the active surveillance period of 45 months, 463 207 units of blood and blood components were transfused at the 2 centers, and 89 TRALI cases were identified (Figure 1). Seventy cases received one or more high plasma volume blood products. Of the 89 TRALI cases, only one had a major ALI risk factor (sepsis); the septic patient was stable until transfusion precipitated pulmonary edema. Nine had minor ALI risk factors (4 receiving amiodarone,

Table 2. Demographic characteristics of TRALI cases and controls

	TRALI cases (N = 89)	Control subjects (N = 164)
Age in y: mean ± SD	54 ± 20	56 ± 20.2
Age in y: median (Q1-Q3)	57 (47-66)	59 (45-71)
Age, y		
≤ 19	9 (10)	9 (6)
20-29	4 (5)	12 (7)
30-39	3 (3)	14 (9)
40-49	12 (14)	14 (9)
50-59	21 (24)	37 (23)
60-69	23 (26)	31 (19)
≥ 70	17 (19)	47 (29)
Sex		
Female	45 (51)	73 (45)
Male	44 (49)	91 (56)
Race		
American Indian/Alaska	1 (1)	2 (1)
Asian	4 (5)	10 (6)
Black or African American	4 (5)	6 (4)
Native Hawaiian/Pacific Islander	1 (1)	1 (1)
Unknown or not reported	16 (18)	22 (13)
White	63 (71)	123 (75)
Ethnicity		
Hispanic	7 (8)	11 (7)
Not Hispanic	70 (79)	124 (76)
Not reporting ethnicity	12 (14)	29 (18)
Hospital		
Mayo Clinic	45 (51)	85 (52)
UCSF	44 (50)	79 (48)
Transfusion strata		
High (≥ 10 units)	30 (34)	48 (30)
Medium (3-9 units)	32 (36)	63 (38)
Low (1-2 units)	27 (30)	53 (32)
Patient location at time of transfusion		
Floor	14 (16)	54 (33)
Hematology Oncology floor	4 (5)	20 (12)
ICU	27 (30)	29 (18)
OR/PACU	43 (48)	53 (32)
Outpatient	1 (1)	8 (5)

Values are no. (%), unless otherwise specified.

3 disseminated intravascular coagulation, 1 after lung resection, 1 acute central nervous system injury/stroke). Of the 89 TRALI cases, only 40 (45%) were reported to the blood banks as a transfusion reaction. Five cases were identified after transfusion reaction reports only, as an ABG was ordered more than 12 hours after blood issue or a FiO₂ was not entered.

TRALI incidence

The annual TRALI incidence (March 1, 2006 to December 31, 2009) decreased from 2.57 (95% confidence interval [CI], 1.72-3.86) per 10 000 units transfused (23 cases/89 321 units) in 2006 to 0.81 (95% CI, 0.44-1.49) per 10 000 units transfused (10 cases/123 731 units) in 2009 (*P* = .002; Figure 2). Reductions from pre- to post-mitigation periods were larger at Mayo (*P* = .014), where TRALI incidence also decreased in patients who received plasma from only male donors, associated with concurrent patient risk factor mitigation.¹⁸ By trend analysis, there was an overall 35% reduction per year (95% CI, 21%-47%, *P* < .0001). In contrast, the decrease in incidence of transfused ALI cases (“possible TRALI”) was not statistically significant (*P* = .38, *p* for trend = 0.78).

Table 3. Descriptive data of components received by control and TRALI patients

	Units received by control patients (N = 164)	Units received by TRALI patients (N = 89)
Total no. of units, mean ± SD	6.43 ± 6.1	11 ± 15.1
Total no. of units, median (range)	3 (1-37)	4 (1-69)
No. of whole blood units, mean ± SD	0.02 ± 0.22	0.13 ± 0.77
No. of whole blood units, median (range)	0.0 (0.0-2)	0.0 (0.0-6)
No. of RBC units, mean ± SD	2.52 ± 2.42	3.57 ± 4.87
No. of RBC units, median (range)	2 (0.0-12)	2 (0.0-25)
No. of apheresis platelets, mean ± SD	0.49 ± 0.779	0.91 ± 1.42
No. of apheresis platelets, median (range)	0.0 (0.0-3)	0.0 (0.0-7)
No. of plasma units, mean ± SD	2.86 ± 4.35	4.69 ± 7.24
No. of plasma units, median (range)	0.0 (0.0-21)	2 (0.0-40)
No. of cryoprecipitate doses, mean ± SD	0.53 ± 2.42	1.66 ± 4.63
No. of cryoprecipitate doses, median (range)	0.0 (0.0-20)	0.0 (0.0-20)
Age of RBC units, mean ± SD (N)	21.5 ± 11 (N = 413)	21.8 ± 11.4 (N = 318)
Age of RBC units, median (range)	20 (3-42)	21 (4-42)
Age of platelet units, mean ± SD (N)	4.91 ± 1.11 (N = 81)	4.99 ± 1.36 (N = 81)
Age of platelet units, median (range)	5 (2-7)	5 (2-7)

In multivariate analysis, the risk of increased no. of involved units was accounted for by the antibody and patient risk factors shown in Table 12 ("Risk of greater number of transfusions").

Incidences of TRALI by transfused patient-days (number of unique patients transfused per 24 hours) in 2006 versus 2009 were 7.46 (95% CI, 4.99-11.19) versus 2.29 (95% CI, 1.26-4.22 per 10 000 patient-days, $P = .002$).

Case-control study

The demographic characteristics (March 1, 2006 to August 31, 2009) of the 89 cases and 164 controls are in Table 2. Descriptive data show more units were transfused to TRALI patients (Table 3). Contents in units transfused to TRALI and control patients are described in Tables 4 to 6. Components received by TRALI patients are in Table 7. Statistically significant univariate patient factors are in Table 8, statistically significant univariate transfusion

Table 4. Descriptive data of antibodies in units transfused to TRALI and control patients

Antibody	Units received by control patients (N = 1054)	Units by TRALI patients (N = 976)
Anti-HNA, positive with at least one granulocyte panel cell by GIFT	6.4% (55/860)	7.8% (56/714)
Anti-HNA, with specificity	1.7% (15/860)	1.7% (12/714)
Anti-HLA-class I, cognate (MFI > 300)	11.6% (108/930)	12.3% (96/782)
Anti-HLA-class II, cognate (MFI > 300)	5.8% (54/930)	7.8% (61/782)

In 12 TRALI units, there were antibodies to HNA-1a, -1b, or -3a. In 15 control units, there were antibodies to HNA-1a, -1b, -1c, -2a, -3a, or 4a. Using the MFI cut-off of > 300 for determination of anti-HLA specificity, the most common cognate anti-HLA-class I specificities were A2 (15 TRALI units, 11 control units) and B7 (10 TRALI units, 10 control units). The most common DQ specificities were DQ7 (7 TRALI units, 13 control units) and DQ8 (9 TRALI units, 11 control units). The most common DR specificity was DR52 (9 TRALI units, 12 control units).

Table 5. Descriptive data of lysophosphatidylcholine (lysoPC) measured in units received by control patients and TRALI patients

	Units received by control patients (N = 536)	Units received by TRALI patients (N = 419)
LysoPC concentration, M, mean ± SD	128 ± 78.3	142 ± 80.6
LysoPC concentration, M, median (range)	137 (0.2-453)	152 (1-367)

LysoPC concentration was the sum of the concentrations of the 16:0 and 18:0 fatty acid moieties. In risk factor analysis, the sum of lysoPC quantities among all units received by a patient was a statistically significant risk factor in univariate analysis (Table 9) but not in multivariate analysis.

factors are in Table 9, and statistically insignificant factors including RBC storage age are in Table 10. Univariate analyses of anti-HLA are in supplemental Table 8 and anti-HNA in supplemental Table 9. Cognate anti-HLA specificities are in supplemental Table 10. The transfusion and patient risk factors with independent predictive value by multivariate analysis are in Tables 11 and 12.

Risk of greater number of transfusions

Initial estimates showed that increased number of transfusions were associated with increased risk, with OR of 4.5 (95% CI, 2.4-8.4, $P < .0001$) for 3 to 9 versus 1 to 2 units, and an additional OR of 1.32 (95% CI, 1.23-1.42, $P < .0001$) per unit beyond 9. To determine whether the risk of greater number of units could be explained by transfusion and patient risk factors we identified, we controlled for these factors in the primary multivariate model (Table 12) and found that the risk of even 10 or more units became small and was no longer statistically significant at OR of 1.05 (95% CI, 0.91-1.20) per unit more than 9 ($P = .53$).

RBC unit storage age

We found evidence against longer storage of leuko-reduced RBC units being an important risk (Table 10). In multivariate models, estimated ORs for older versus fresher RBC units were in the protective directions for all age cut-offs (ORs, 0.80-0.92).

Plasma from female donors

Plasma (including plasma in whole blood) from female donors has been a focus of TRALI risk, and we found this to be a strong risk factor in multivariate analysis controlling for patient risk factors only (OR = 4.5, 95% CI, 1.85-11.2, $P = .001$; Table 11). Replacing plasma from female donors with antibody risk factors in the multivariate model, we found that 2 antibody measures were strong risk factors: quantity of cognate anti-HLA-class II (MFI > 1500) and volume of units anti-HNA positive by GIFT (Table 12). When female donor plasma and the 2 antibodies were in the same model, female donor plasma risk was smaller and no longer statistically significant (OR = 2.4, 95% CI, 0.87-6.9, $P = .09$).

Specific HLA antibody

Concerning the importance of cognate HLA antibody, total quantity of cognate anti-HLA-class II (MFI > 1500) was associated with risk in the multivariate analysis (Table 12). However, for anti-HLA-class II (MFI > 1500), total quantity, strongest MFI in any unit, and largest quantity in any unit were all collinear and highly predictive (OR = 3.2-3.5, $P = .0035$ -.0052), and which is most important cannot be reliably determined from our data. In contrast, when controlled for cognate anti-HLA-class II (MFI > 1500), no

Table 6. Descriptive data of neutrophil priming activity in transfused units

CD11b or CD66b up-regulation	Units received by control patients (N = 532)	Units received by TRALI patients (N = 321)
% cells positive for CD11b, mean ± SD	9.02 ± 12.7	10.8 ± 14.7
% cells positive for CD11b, median (range)	3.8 (0.0-78.4)	4.1 (0.0-73.7)
% cells positive for CD11b or CD66b, mean ± SD	5.94 ± 8.4	5.12 ± 6.36
% cells positive for CD11b or CD66b, median (range)	2.4 (0.0-61.9)	2.8 (0.0-49.9)

In risk factor analysis, total quantities of CD11b up-regulation (% positive cells × plasma volume) in all units received by a patient were a statistically significant risk factor in univariate analysis (Table 9) but not in multivariate analysis.

substantial risk was associated with the quantity of noncognate anti-HLA-class II (MFI > 1500, OR = 0.41, 95% CI, 0.10-1.59, *P* = .19).

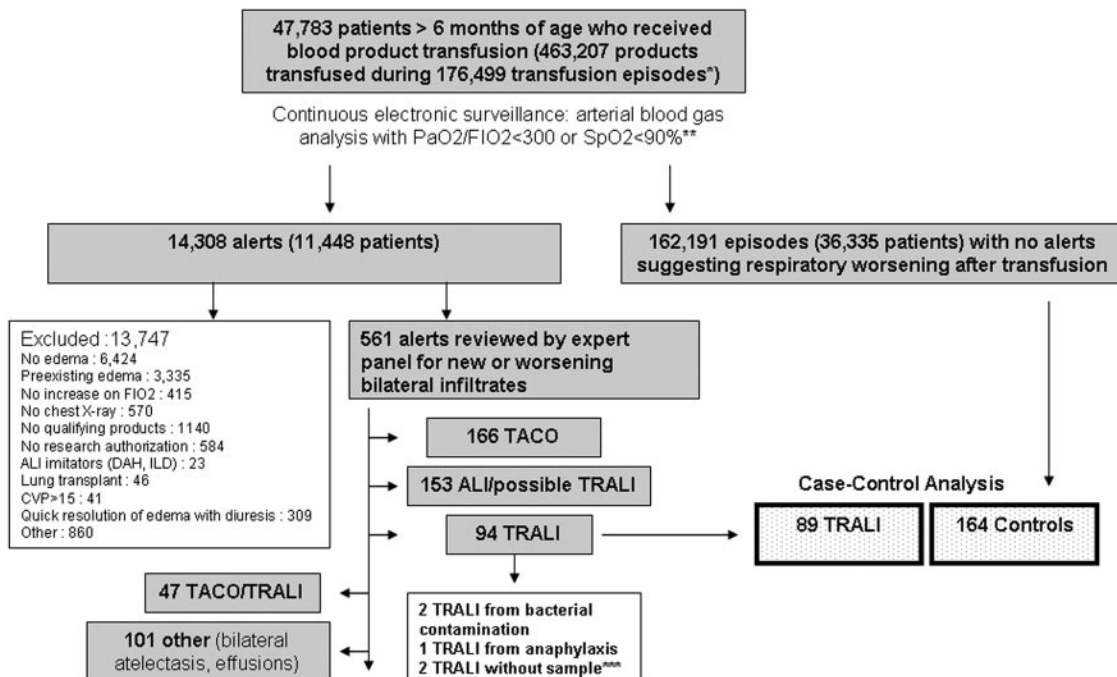
Regarding the importance of class I cognate HLA antibody, no substantial risk was associated with the presence of any cognate anti-HLA-class I (MFI > 2500, OR = 1.12, 95% CI, 0.20-6.20, *P* = .90), when controlled for cognate anti-HLA-class II (MFI > 1500).

With regard to the relative importance of strong (MFI > 1500) versus weak (MFI 300-1500) antibody, no substantial risk was associated with the presence of any weak cognate anti-HLA-class II (MFI 300-1500, OR = 0.32, 95% CI, 0.09-1.20, *P* = .09), when controlled for cognate anti-HLA-class II (MFI > 1500).

Regarding cognate HLA antibody specificities (supplemental Table 10), no single strong cognate HLA class I or II specificity was statistically significant when added to the primary multivariate model (Table 12) as an any versus no dichotomous predictor, although many were too rare to be evaluated individually.

HLA antibody detected by screening test

Among study units, 84% (1712 of 2030) were tested for HLA antibody by the screening test. To facilitate applying the findings to clinical practice, we investigated what level of antibody (NBG ratio) found by the anti-HLA donor screening test would pose a significantly increased risk for transfusion recipients. After testing multiple assay thresholds (in models that did not include the cognate HLA class II variable), we found that a larger volume of strong anti-HLA-class II (NBG ratio > 27.5) increased risk (OR = 1.92 per 100 mL, 95% CI, 1.08-3.4, *P* = .03), a threshold equivalent to greater than 5 SDs in a cohort of nontransfused males.¹⁵ However, similar to the specific anti-HLA antibodies, the volume of weak anti-HLA-class II positive on the screening test (NBG ratio, 2.2-27.5) was not associated with risk (OR = 0.81 per 100 mL, 95% CI, 0.34-1.93, *P* = .63), and the volume of anti-HLA-class I did not appear to increase risk, even for strong antibody (NBG ratio > 59.3, OR = 0.98 per 100 mL, 95% CI, 0.46-2.1, *P* = .97).



* Transfusion episode was defined as transfusion during 24hour period
 ** If PaO2 was not reported – in the operating room
 *** After Sample collection completed 8/31/2009

Figure 1. Enrollment of TRALI cases and controls at 2 academic centers (2006-2009). All RBC and platelet units (all collected by apheresis) were leuko-reduced. To determine risk factors, 89 TRALI cases and 164 controls that occurred during the case-control study period from March 1, 2006 to August 31, 2009 were included. DAH indicates diffuse alveolar hemorrhage; ILD, interstitial lung disease; CVP, central venous pressure; and TACO, transfusion associated circulatory overload.

Table 7. All components received during or within 6 hours in 89 cases of TRALI

Components	No. of cases	% of cases
Only whole blood	1	1
Only plasma	13	15
Only platelets	5	6
Only RBCs	20	22
Mixed components, no plasma	4	4
Mixed components, with plasma	46	52
Total	89	100

HNA antibody

Among study units, 78% (1574 of 2030) were tested for anti-HNA by GAT and GIFT. Patients were not tested for HNA type, except for the 2 recipients of anti-HNA-3a. By multivariate analysis, plasma volume of all GIFT-positive units received was a predictor of risk (OR = 1.71 per 100 mL, 95% CI, 1.18-2.5, $P = .004$; Table 12). GAT was not a statistically significant predictor when controlled for GIFT. Anti-HNA with defined specificity was rare (Table 4), including anti-HNA-3a. Of the 1574 tested units, only 2 (0.1%) contained anti-HNA-3a: 1 in an RBC and 1 in a plasma unit. A TRALI patient received this RBC unit and other units that contained strong cognate anti-HLA-class II; a control patient received the plasma unit. Both recipient genotypes were homozygous for HNA-3a (courtesy of Dr Brian Curtis, Blood Center Wisconsin). GIFT strength could not be quantified (see supplemental Discussion).

Receipt of antibody predictors by patients

Among the 89 TRALI patients, 52% received one or both antibody predictors (10% received cognate anti-HLA-class II with MFI > 1500, 12% received anti-HNA positive by GIFT, and 30% received both).

Bioreactive substances

Bioreactive substances were significant by univariate analysis (Table 9), but when added to the primary multivariate model (Table 12), the total amounts of MICA antibody and candidate bioreactive molecules were not statistically significant. For bioreactive substances, half of the units were not returned, and missing data required multiple imputation ("Statistical methods").

Patient risk factors

On univariate analysis, we identified patient risk factors that increased the risk of TRALI or were protective (Table 8). However, on multivariate analysis, the protective factors dropped out of the model, leaving independent patient risk factors of shock, liver surgery (mainly transplantation), chronic alcohol abuse, positive fluid balance, peak airway pressure greater than 30 cm H₂O if mechanically ventilated before transfusion, and current smoking (Tables 11 and 12). The patient's plasma IL-8 level measured before transfusion was also a predictor of risk by multivariate analysis.

Discussion

The major findings of this first study of TRALI by active surveillance of the general transfusion population can be summarized as follows. First, implementation of a predominantly male plasma supply was associated with reduced incidence of TRALI determined by active surveillance, thus supporting the effective-

ness of this approach. Second, 2 predictive transfusion risk factors identified were the quantities of strong cognate anti-HLA-class II and volumes of anti-HNA in the blood units. No substantial risk was found for noncognate or weak cognate anti-HLA-class II, or anti-HLA-class I. Third, the data support using a cut-off of NBG ratio more than 27.5 for screening of anti-HLA-class II in female apheresis platelet donors. Fourth, the combination of transfusion and patient risk factors appeared to explain most of the risk of developing TRALI known to be associated with multiple transfusions.⁵ Fifth, we found evidence against longer RBC storage age being an important risk factor.⁹

We found that receipt of plasma (including whole blood) from female donors is a strong risk factor, and reduction of this risk factor was concurrent with a decrease in TRALI incidence from approximately 1:4000 units to approximately 1:12 000 units. The premitigation incidence of approximately 1:5000 units found in 2006 was close to the approximately 1:5000 units found by a careful study in 1985 at the Mayo Clinic where a transfusion team performed and monitored transfusions,⁶ but 10-fold higher than the 2005 premitigation incidence of approximately 1:40 000 units distributed found by passive surveillance (26.3 cases in 10⁶ units).¹⁹ In our study, other factors may have contributed to the decrease in incidence (eg, institutional improvements in critical care delivery that reduced patient risk factors we found that appear to render patients susceptible to TRALI, such as improving treatment of septic shock and decreasing high peak airway pressure > 30 cm H₂O while being mechanically ventilated).¹⁸ Decreases in TRALI after conversion to plasma from predominantly male donors have been reported by passive surveillance studies from the United Kingdom,²⁰ the FDA,¹ and the American Red Cross.²¹ No similar decrease in "possible TRALI" incidence after implementation of the TRALI mitigation strategy suggests that plasma from alloimmunized donors is not a strong risk factor in "possible TRALI."

Although units transfused to TRALI and control patients were similar in terms of percent positive for antibody (Table 4), TRALI patients received more units of every component (Table 3) and thus

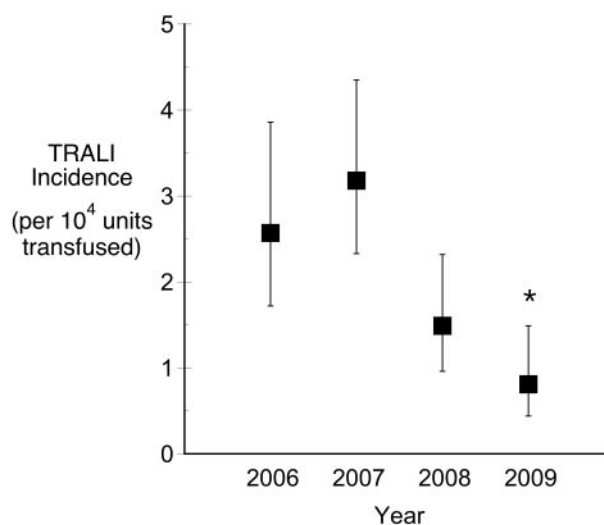


Figure 2. TRALI incidence by year at 2 academic medical centers (2006-2009). Reduction of high-risk plasma was implemented from 2007 to 2008. In 2006, neither center had started TRALI mitigation. In 2009, both centers had completed TRALI mitigation. The annual TRALI incidence decreased from 2.57 (95% CI, 1.72-3.86) per 10 000 units transfused (23 cases/89 321 units) in 2006 to 0.81 (95% CI, 0.44-1.49) per 10 000 units transfused (10 cases/123 731 units) in 2009. * $P = .002$. There was an estimated 35% reduction in TRALI per year by trend analysis (95% CI, 21%-47%, $P < .0001$).

Table 8. Univariate patient risk factors for TRALI

	No. TRALI/no. without risk factor (%)	No. TRALI/no. with risk factor (%)	OR	Lower 95% CI	Upper 95% CI	P
Medical factors						
≥ 10 units, any component (per unit > 9)	NA	NA	1.17	1.05	1.31	.006
Shock before transfusion	50/184(27.2)	36/69(56.5)	4.3	2.2	8.4	< .001
Fluid balance pre-TRALI (increment per liter)	NA	NA	1.17	1.08	1.28	< .001
Acute renal failure	66/213(31.0)	23/41(57.5)	2.5	1.22	5.2	.010
Smoking history (current vs other)	67/212(31.6)	18/36(50.0)	1.99	0.95	4.2	.07
Peak airway pressure > 30 cmH ₂ O if mechanically ventilated	73/230(31.7)	16/21(69.6)	5.6	2.1	14.9	< .001
Chronic alcohol abuse	75/229(32.8)	12/18(88.7)	3.0	1.07	8.7	.04
Severe liver disease	68/217(31.3)	21/36(58.3)	2.9	1.34	6.2	.007
Surgery type, reference group = no surgery			1			
Liver	74/233(31.8)	15/20(75.0)	12.1	3.4	43.1	< .001
Spine	78/230(33.9)	11/23(47.8)	3.9	1.32	11.3	.013
Cardiac	77/227(33.9)	12/26(46.2)	3.3	1.21	9.2	.02
Vascular	85/243(35.0)	4/10(40.0)	2.8	0.65	12.0	.17
Other	67/178(37.6)	22/75(29.3)	1.40	0.69	2.8	.35
Markers of inflammation before transfusion						
Log ₁₀ IL-6	NA	NA	1.65	1.04	2.6	.03
Log ₁₀ IL-8	NA	NA	1.79	1.02	3.1	.04
Biologically plausible protective factors						
Immunosuppression	66/166(39.8)	22/85(25.9)	0.52	0.28	0.95	.03
Leukemia or lymphoma	82/216(38.0)	6/36(16.7)	0.32	0.12	0.83	.02
Pre-TRALI platelet count < 50 × 10 ⁹ /L	72/176(40.9)	10/50(20.0)	0.36	0.17	0.79	.010
Pre-TRALI WBC count < 0.5 × 10 ⁹ /L	81/221(36.7)	2/15(13.3)	0.32	0.07	1.51	.15
Patient illness severity as indicated by location of the patient at the time of transfusion*						
Intensive care unit (reference group)			1			
Operating room or postoperative recovery unit	46/157(29.3)	43/96(44.8)	1.05	0.51	2.2	.90
Floor	75/185(40.5)	14/68(20.6)	0.22	0.09	0.54	< .001
Hematology-oncology floor	85/229(37.1)	4/24(16.7)	0.19	0.05	0.68	.010
Outpatient	88/244(36.1)	1/9(11.1)	0.11	0.012	1.01	.05

NA indicates not applicable because the factor is a continuous variable.
 *May or may not include the first transfusion.

probably received larger quantities/volumes of antibody. Multivariate analysis confirmed that antibody strength and quantity/volume are important (Table 12).

Regarding the relative importance of class I versus class II HLA antibody, we found class II to be more important. The association of anti-HLA-class II with TRALI was first reported by Kopko et al.²² Case series have reported that cognate anti-HLA-class II was the most frequent antibody implicated in TRALI.^{23,24} This predominance occurs despite the fact that frequencies of class I (10%) and class II (12%) antibodies are comparable in female donors.²⁵ The greater importance of anti-HLA-class II appears to also be true in renal transplantation.²⁶

Regarding anti-HLA-class I, we found evidence against anti-HLA-class I being an important risk, even for strong cognate antibody with MFI more than 2500 (OR = 1.12, 95% CI, 0.20-6.20, P = .90). Others have reported similar results⁷ and similar conclusions.²⁷ Although the estimated risk for class I was very small and not statistically significant, we cannot be certain that there is absolutely no risk, as the upper confidence bound of 6.20 does not preclude risk. There are reports that cognate anti-HLA-class I can be associated with TRALI.^{23,24,28} However, retrospective studies have found that this is rare.^{29,30}

Regarding cognate versus noncognate HLA antibody, we found evidence against substantial risk from noncognate antibody. This finding argues against the hypothesis that noncognate HLA antibody is a marker for blood donors who make other antibodies that increase TRALI risk.

Volume of anti-HNA positive by GIFT was a strong predictor (Table 12). The GIFT detects antibodies to HNA, antibodies to unknown HNA, and anti-HLA-class I. Because we found that anti-HLA-class I did not appear to produce substantial risk, the risk detected by the GIFT is probably the result of anti-HNA. Because specific anti-HNA was rare, we could not examine the contribution of anti-HNA specificities. There may be unidentified HNA antigens that may be important.

The 2 antibody predictors in Table 12 are applicable to all component types. For example, for the same cognate anti-HLA-class II with MFI more than 1500, the estimated risk is 3.2 times greater in 200 mL of plasma (plasma, apheresis platelets) than in 20 mL of plasma (RBCs, cryoprecipitate) because the OR is 3.2 per 10-fold increase in amount transfused. For anti-HNA positive by GIFT, the estimated risk is 2.6 times greater for 200 mL than for 20 mL of plasma because the OR is 1.71 per 100-mL increase.

Whether longer RBC storage is associated with increased risk for lung injury and mortality is considered the most critical issue currently facing transfusion medicine.⁹ In this prospective case-control study, we found evidence against longer storage of leuko-reduced RBC units being an important risk for TRALI.

The risks associated with bioreactive substances were not statistically significant in the multivariate model (Table 12), similar to the results in a study of cardiac surgery patients.⁸ This result was surprising, given many pioneering basic studies by Silliman et al indicating that bioreactive substances are important in the development of TRALI.³¹⁻³⁴ However, the CIs of the odds ratios

Table 9. Univariate transfusion risk factors for TRALI

Transfusion factor assessed for each patient	OR	Lower 95% CI	Upper 95% CI	P
Blood or blood component(s) received				
Plasma from female donor	3.0	1.44	6.3	.004
Plasma from previously pregnant female donor	2.2	1.08	4.6	.03
Platelets from previously pregnant female donor	2.5	1.19	5.4	.02
Cryoprecipitate from female donor	5.5	1.41	21.4	.014
Cryoprecipitate from previously pregnant female donor	5.8	1.49	22.5	.014
MICA antibody, sum of amounts in all units received				
MICA antibody	1.28	1.08	1.53	.006
LysoPC and cytokines: sum of amounts (concentration × volume) in all units received				
LysoPC	1.27	1.07	1.51	.006
IL1ra	1.12	1.01	1.24	.04
IL1b	1.19	1.03	1.36	.02
VEGF	1.04	1.00	1.08	.03
Neutrophil priming activity: sum of amounts (% neutrophils positive for CD11b up-regulation × volume) in all units received				
CD11b up-regulation	1.04	1.01	1.07	.008

Numbers of patients who received or did not receive this transfusion risk factor are not available because multiple imputation was used for units with missing data ("Statistical methods: risk factor analysis"). Other not statistically significant univariate transfusion risk factors are described in Table 10. Univariate antibody factors are not described in this table, but univariate anti-HLA factors are described in supplemental Table 8, and anti-HNA factors are described in supplemental Table 9.

in our results were wide, and the upper bounds would correspond to substantial risk. Thus, we cannot conclude that these substances do not carry risk. Future clinical studies should test for these important potential mediators in the setting of ongoing TRALI mitigation.

Why do some patients develop TRALI and others who receive blood from the same donor do not? We found that cognate antibody matters, and patients who developed TRALI may have received cognate antibody and others did not. However, cognate versus noncognate antibody is not the only reason, as several look-back studies have found patients who received cognate antibody but did not develop TRALI.^{29,30,35} We found 4 additional factors that influence why some patients develop TRALI and others do not: first, the volume of antibody transfused, second, the HLA class of the cognate antibody, third, the strength of anti-HLA-class II, and fourth, the presence or absence of patient factors that increase the risk and lowers the threshold³⁶ for TRALI.

Approximately half of the TRALI patients received cognate anti-HLA-class II (MFI > 1500) or anti-HNA. Some remaining cases may have received transfusion factors for which we found evidence against substantial risk, but the upper bounds of the CIs still included the possibility of some risk. Some cases may have received other transfusion factors we did not study (supplemental Discussion). In some cases with patient risk factors, it may be possible that transfusion was coincidental.

The diverse patient-associated risk factors we found for TRALI in this study are consistent with the known underlying comorbidities that predispose and lower the threshold for ALI, thus supporting the validity of this study. Shock results in tissue injury,³⁷ perhaps predisposing to TRALI through priming of the recipient's neutrophils. Chronic alcohol abuse increases risk, probably because of reduced levels of the antioxidant glutathione in the lung,³⁸ reduced phagocytosis of apoptotic cells, and the resulting enhanced pulmonary inflammatory response.³⁹ Patients with intravascular volume overload are more likely to manifest clinical pulmonary edema when there is ALI.⁴⁰ Previous studies have documented the risk for developing ALI with peak airway pressure greater than 30 cm H₂O⁴¹ and current smoking.⁴²

We found higher IL-8 in patients before transfusion of involved units increased risk. Higher IL-8, a marker of inflammation and increased mortality risk,⁴³ may prime neutrophils and the lung endothelium. Acute contemporaneous events that increase inflammation and tissue injury could be "first hits" as first suggested by Silliman et al.³¹ Experimental models of TRALI have shown that host inflammation may be necessary to produce ALI before challenge with cognate antibody.^{32,44} Inflammation (first hit) may up-regulate expression of HLA class II antigens on activated classic antigen-presenting cells (monocytes,⁴⁵ macrophages, dendritic cells), activated neutrophils,⁴⁶ and activated lung endothelial cells,⁴⁷ and exposure to large quantities of strong cognate anti-HLA-class II may then lead to ALI (second hit).⁴⁸

Liver surgery (transplantation) was a patient risk identified, even when controlled for severe liver disease, volume of transfusions, and alcohol abuse.^{49,50} Cardiac surgery⁸ and spine surgery were not significant factors in multivariate analysis.

The primary strength of this study is that it is the largest prospective, case-controlled study of TRALI identified by active surveillance in a general population of transfused patients at academic centers, and a large number of biologically plausible risk factors were studied. Limitations include possible missed TRALI patients who had no FiO₂ data available or an ABG was obtained after 12 hours of blood issue, and no report of the reaction was made to the blood bank. The wide CIs of the odds ratios, because of missing data, the decrease in TRALI incidence, and possibly differences in storage times of units transfused to cases and controls, limited the strength of evidence that this study could provide, particularly for negative findings (eg, bioreactive substances). In addition, we could not assess the effect of measures to reduce transfusion of platelets from alloimmunized donors because it was partially implemented late in this study.

In conclusion, this prospective study indicates that TRALI incidence, as determined by active surveillance, decreased after reduction of transfusion of plasma from female donors. The decrease in TRALI incidence was probably the result in part of reduced transfusion of strong cognate HLA class II antibodies and HNA antibodies to patients who are susceptible to ALI, although decreases in patient risk factors probably also played a role. To

Table 10. Univariate transfusion factors (*P* > .05)

Transfusion risk factor assessed for each patient	OR	Lower 95% CI	Upper 95% CI	<i>P</i>
Blood or blood component received				
RBCs from female donor	0.62	0.35	1.08	.09
RBCs from previously pregnant female donor	0.79	0.45	1.38	.40
Cytokines: sum of amounts (concentration × volume) in all units received				
IL-6	1.14	0.997	1.30	.06
IL-8	1.26	0.96	1.63	.09
GM-CSF	1.00	0.999	1.00	.52
IFN-γ	1.24	0.99	1.56	.06
TNF-α	1.10	0.96	1.27	.18
Soluble CD40 ligand (sCD40L)	1.06	0.97	1.16	.22
Platelet factor 4 (PF4)	1.06	0.98	1.14	.14
Neutrophil priming activity: sum of amounts (activity × volume) in all units received				
CD11b/CD66b up-regulation	1.00	0.95	1.04	.82
No. of leuko-reduced RBC units received by each patient, by RBC unit storage age				
RBC units vs other units	1.02	0.90	1.15	.77
No. of RBC units > 7-day-old vs other RBC units	1.02	0.76	1.37	.88
No. of RBC units > 14 days vs other RBC units	1.03	0.87	1.23	.72
No. of RBC units > 21-day-old vs other RBC units	1.13	0.95	1.34	.16
No. of RBC units > 28-day-old vs other RBC units	1.03	0.81	1.31	.79
No. of RBC units > 35-day-old vs other RBC units	0.99	0.76	1.29	.95
Median age of all RBCs vs other RBC units	1.01	0.99	1.03	.49
Mean age of all RBC units	1.01	0.99	1.03	.41
Maximum age among all RBC units	1.01	0.99	1.03	.26
Apheresis platelet unit storage age				
Platelets > 3-day-old vs other platelets	0.67	0.27	1.68	.39
Platelets > 4-day-old vs other	1.24	0.72	2.1	.45
Platelet median unit age	1.07	0.96	1.20	.20
Platelet mean unit age	1.07	0.96	1.2	.22
Platelet maximum unit age	1.07	0.96	1.2	.20

The transfusion univariate risk factors in this table were not statistically significant. Numbers of patients who received or did not receive this transfusion risk factor are not available because multiple imputation was used for units with missing data ("Statistical methods: risk factor analysis").

further reduce TRALI risk, our clinical evidence supports consideration of screening donors for strong HLA class II antibodies¹⁵ and the development of high-throughput GIFT methods to screen for antibodies to known and unknown human neutrophil antigens. Importantly, reduction of modifiable patient risk factors should also reduce the risk for developing TRALI.¹⁸

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Authorship

Contribution: P.T., O.G., E.L.M., M.A.M., P.B., M.R.L., R.B.W., R.H., C.A.L., and M.A.G. conceived and designed the study; G.W.,

Table 11. Receipt of plasma from female donors controlled for recipient factors

Transfusion risk factor	OR	Lower 95% CI	Upper 95% CI	<i>P</i>
Transfusion risk factor				
Receipt of any female donor plasma or whole blood	4.5	1.85	11.2	.001
Recipient risk factors				
Chronic alcohol abuse	3.8	1.03	13.8	.045
Fluid balance before transfusion (increment per liter)	1.2	1.05	1.3	.006
Peak airway pressure > 30 cmH ₂ O within 12 hours after intubation before transfusion	2.8	0.89	8.5	.08
Shock before transfusion	2.9	1.32	6.4	.008
Current smoker vs never or former smoker	3.3	1.32	8.2	.01
Liver surgery (transplantation)	3.0	0.78	11.8	.11

See Table 8 for numbers of patients for patient risk factors. Numbers of patients who received or did not receive this transfusion risk factor are not available because multiple imputation was used for units with missing data ("Statistical methods: risk factor analysis").

Table 12. Primary multivariate model of TRALI risk factors: antibodies transfused to the recipient controlled for recipient risk factors

	OR	Lower 95% CI	Upper 95% CI	P
Transfusion risk factors among all transfusions to each patient				
Total quantity of cognate anti-HLA-class II (MFI > 1500) per 10-fold increase	3.2	1.52	6.7	.002
Total volume of anti-HNA positive by GIFT among all units, per 100-mL increase	1.71	1.18	2.5	.004
Recipient risk factors				
Chronic alcohol abuse	5.9	1.22	28.3	.028
Fluid balance before transfusion (increment per liter)	1.15	1.02	1.29	.024
Peak airway pressure > 30 cmH ₂ O within 12 hours after intubation before transfusion	3.6	1.01	13.1	.048
Shock before transfusion	4.2	1.69	10.6	.002
Current smoker vs never or former smoker	3.4	1.22	9.7	.020
Liver surgery (transplantation)	6.7	1.25	35.7	.027
IL-8 concentration before transfusion, per 10-fold increase	3.0	1.30	6.8	.018

See Table 8 for numbers of patients for patient risk factors. Numbers of patients who received or did not receive this transfusion risk factor are not available because multiple imputation was used for units with missing data ("Statistical methods: risk factor analysis"). The patient's plasma IL-8 level measured before transfusion was also a predictor of risk in multivariate analysis. Because this variable was missing for 50 patients, reducing the set of analyzed patients to accommodate this variable disrupted the estimates of other risk factors, and so this was not included in the primary model but listed at the end of the table.

M.K., R.S.R., R.S., D.L., and P.W. acquired data; P.B., B.G., P.T., M.A.M., M.R.L., and R.B.W. analyzed and interpreted data; P.B. and B.G. performed statistical analysis; P.T., P.B., O.G., E.L.M., M.A.M., M.R.L., R.B.W., R.H., and C.A.L. obtained funding; G.W., M.K., R.S.R., R.S., D.L., and P.W. provided technical or material support; P.T. supervised the study; O.G. supervised the Mayo Clinic site; J.L.W. supervised collection of blood donor samples and data at Mayo Clinic; N.H. supervised collection of blood donor samples and data at Blood Centers of the Pacific; M.J.G. supervised the laboratory testing for HLA typing and antibody; P.J.N. supervised data download of and assisted in the

analyses of HLA antibody screening and single-antigen bead test results; D.M. supervised the testing for antibody to human neutrophil antigens; C.A.L. supervised laboratory testing for cytokines and neutrophil priming activity; and all authors drafted the manuscript and critically revised the manuscript for important intellectual content.

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