Transfusion-related Acute Lung Injury in the Critically Ill
Prospective Nested Case-Control Study

Ognjen Gajic[1,4], Rimki Rana[1], Jeffrey L. Winters[2], Murat Yilmaz[1], Jose L. Mendez[1], Otis B. Rickman[1], Megan M. O'Byrne[3,4], Laura K. Evenson[1,4,5], Michael Malinchoc[3,4], Steven R. DeGoey[2], Bekele Afessa[1,4], Rolf D. Hubmayr[1,4], and S. Breanndan Moore[2]

[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, [2]Division of Transfusion Medicine, Department of Laboratory Medicine and Pathology, and [3]Division of Biostatistics, Department of Health Sciences Research, [4]Mayo Epidemiology and Translational Research in Intensive Care (METRIC), and [5]Department of Nursing, Mayo Clinic College of Medicine, Rochester, Minnesota

***Rationale*: Acute lung injury (ALI) that develops 6 hours after transfusion (TRALI) is the leading cause of transfusion-related mortality. Several transfusion characteristics have been postulated as risk factors for TRALI, but the evidence is limited to retrospective studies.**

***Objectives*: To compare patient and transfusion risk factors between patients who do and do not develop ALI.**

***Methods*: In this prospective cohort study, consecutive transfused critically ill patients were closely observed for development of ALI. Donor samples were collected from the transfusion bags. Risk factors were compared between patients who developed ALI after transfusion and transfused control patients, matched by age, sex, and admission diagnosis.**

***Measurements and Main Results*: Seventy-four of 901 transfused patients developed ALI within 6 hours of transfusion (8%). Compared with transfused control subjects, patients with ALI were more likely to have sepsis (37 vs. 22%, $P = 0.016$) and a history of chronic alcohol abuse (37 vs. 18%, $P = 0.006$). When adjusted for patient characteristics, transfusion of plasma from female donors (odds ratio [OR], 5.09; 95% confidence interval [95% CI], 1.37–18.85) rather than male donors (OR, 1.60; 95% CI, 0.76 to 3.37), number of pregnancies among the donors (OR, 1.19; 95% CI, 1.05 to 1.34), number of donor units positive for anti-granulocyte antibodies (OR, 4.85; 95% CI, 1.32–17.86) and anti–HLA class II antibodies (OR, 3.08; 95% CI, 1.15–8.25), and concentration of lysophosphatidylcholine in the donor product (OR, 1.69; 95% CI, 1.10 to 2.59) were associated with the development of ALI.**

***Conclusions*: Both patient and transfusion risk factors determine the probability of ALI after transfusion. Transfusion factors represent attractive targets for the prevention of ALI.**

Keywords: fresh-frozen plasma; platelet transfusion; pulmonary edema; female; blood donors

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Transfusion-related acute lung injury is the leading cause of transfusion-related mortality and is believed to be widely underrecognized. Animal experiments and uncontrolled clinical studies suggest that specific transfusion factors play an important mechanistic role.

What This Study Adds to the Field

When a standardized definition was applied in a prospective cohort of critically ill patients, acute lung injury commonly occurred within 6 hours of transfusion. Plasma from alloimmunized donors predicted acute lung injury development, raising important implications for acute lung injury prevention.

Since the original description (1), transfusion-related acute lung injury (TRALI) has emerged as the most important cause of morbidity and mortality resulting from blood transfusion (2–4), in part because of the decrease in morbidity and mortality from other adverse effects of transfusion. Although the exact etiology is not known, uncontrolled clinical studies and animal models have suggested an important role for donor-derived anti-leukocyte antibodies as well as biological response modifiers accumulated during blood storage (5–9). Anti-leukocyte antibodies (anti–HLA class I, anti–HLA class II, and anti-granulocyte) are known to be commonly present in the plasma of donors exposed to foreign tissue during previous pregnancy or transfusion and have been implicated in multiple case series and case reports (1, 3–5). Indeed, anti-HLA antibodies have been detected in up to 25% of multiparous female donors (10).

TRALI is believed to be underdiagnosed and underreported (4, 5, 11), particularly in critically ill patients who often have multiple risk factors for acute lung injury (ALI). It is possible that current TRALI cases reported to blood banks represent the "tip of the iceberg" and that transfusion factors play a mechanistic role in many more patients with ALI, as one of "multiple hits" required for the full expression of this syndrome (12–14). Indeed, transfusion (massive) has long been recognized as one of the most important ALI risk factors (15). Submassive transfusion of all blood products, and particularly of high plasma volume products, has been identified as an independent risk factor for development of ALI in the critically ill (16, 17). In animal studies, the presence of additional ALI risk factors has been found to modulate both the development and severity of TRALI (multiple-hit hypothesis) (8, 9). The lack of a standardized definition, however, posed a significant limitation on previous clinical studies (3, 4).

Two expert consensus panels have proposed a standardized TRALI definition (3, 4). Using the proposed definition, we reported retrospective data showing a much higher incidence of TRALI in critically ill patients than previously suggested (18, 19). In this prospective study we aimed to determine the incidence, risk factors, and outcome of ALI that develops 0–6 hours after the transfusion of blood products in a cohort of patients admitted to

(*Received in original form February 16, 2007; accepted in final form July 12, 2007*)

Supported by the NHLBI (1 K23 HL087843-01A1) and by a grant from the National Blood Foundation (NBF 2004-05).

Correspondence and requests for reprints should be addressed to Ognjen Gajic, M.D., Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: gajic.ognjen@mayo.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 176. pp 886–891, 2007
Originally Published in Press as DOI: 10.1164/rccm.200702-271OC on July 12, 2007
Internet address: www.atsjournals.org

the medical intensive care unit (ICU). Using a matched case-control design, we compared types of blood products transfused by donor and storage characteristics, the prevalence of anti-leukocyte antibodies (anti-granulocyte, anti–HLA class I, and anti–HLA class II) (1, 5), and the amount of biological response modifiers (IL-8 and lysophosphatidylcholine [LysoPC]) (20) in the samples of donor blood units given to patients who did or did not develop ALI after transfusion.

Some of the results of this study have been previously reported in the form of an abstract (21).

METHODS

In this 2-year prospective cohort study, all patients transfused in a medical ICU of a tertiary care medical center were closely observed for 24 hours after transfusion. (For detailed methods, *see* the online supplement.) The institutional review board approved the study protocol. Patients who refused research authorization were excluded. Monitoring logs reflecting cardiopulmonary function, chest radiographs, and arterial blood gases were reviewed and monitored until 24 hours after each transfusion, allowing for identification of patients who had any worsening of respiratory status 0–24 hours after the transfusion (*see* the online supplement). Expert intensivists, blinded to specific transfusion factors, subsequently reviewed all clinical data and assigned the diagnosis of ALI within 6 hours of transfusion (suspected or possible TRALI) on the basis of the standard clinical definition (4, 22, 23). Interobserver agreement was measured by κ statistics. Two of the reviewers (J.L.M. and O.B.R.) re-reviewed the discordant cases together and resolved any disagreements.

In the nested case-control part of the study, the patient and transfusion factors were compared between patients who developed ALI after transfusion and transfused control patients matched by age $\pm$ 10 years, sex, and admission diagnostic group (19). Predictor variables were grouped as follows:

1. ALI risk factors before transfusion: Severity of illness was determined by calculating Acute Physiology and Chronic Health Evaluation (APACHE) III scores (24) before transfusion. Sepsis and pneumonia were defined according to standard clinical criteria (25, 26). Aspiration was defined as witnessed or suspected aspiration of gastric contents into the airways. Chronic alcohol abuse was defined as a known diagnosis of chronic alcoholism or a previous admission for alcohol detoxification or alcohol withdrawal (27).
2. Transfusion factors: Units given 0–6 hours before the development of ALI (in cases) or 0–6 hours after the beginning of the first transfusion (control subjects) were considered associated units. The exposure time for each control subject was matched to the exposure time of the corresponding case subject (*see* the online supplement). Data on type of blood product, storage age, and donor sex were collected from the institutional transfusion database. Female donors were contacted to obtain pregnancy history unless available from the medical record of the donor. Transfused plasma volume was calculated on the basis of the average plasma content in specific blood products (19). Fresh-frozen plasma (FFP) and platelet transfusions were considered high plasma volume components (28).
3. Donor plasma samples: Throughout the study period transfusion bags and tubing were collected at the end of each transfusion and stored for subsequent testing. Anti–HLA class I and anti–HLA class II antibodies were measured in a multiplexed, microsphere-based flow cytometric assay (LABScreen PRA; One Lambda, Inc., Canoga Park, CA) (29). Anti-granulocyte antibodies were detected by a standard indirect immunofluorescence method (30). LysoPC was measured by high-performance lipid chromatography (31). IL-8 was measured in a standard ELISA (Quantikine HS immunoassay; R&D Systems, Inc., Minneapolis, MN). If an insufficient amount of plasma was obtained from the transfusion bag, antibody (but not LysoPC and IL-8) testing was performed on available donor product or donor sample from a subsequent donation.

Statistical Analysis

Paired parametric and nonparametric testing was used in univariate analyses as appropriate. We anticipated that approximately 80% of case subjects, and 40% of control subjects, will have received at least 1 unit of donor blood that tests positive for anti-leukocyte antibodies (6, 20). It was determined that a 1:1 matched case-control study with 50 case subjects and 50 control subjects would provide more than 80% power to detect a significant difference in the number of positive antibody tests between groups, using a two-sided, $\alpha = 0.05$ level test. Conditional logistic regression was used to compare specific transfusion factors after the adjustment for pretransfusion ALI risk factors. SAS statistical software was used for all analyses (SAS version 9; SAS Institute, Inc., Cary, NC).

RESULTS

We prospectively observed 901 critically ill patients who were transfused in the medical ICU over the 2-year study period (*see* outline of the study in Figure 1). Among 6,588 blood product units transfused during the ICU stay 3,383 (51%) were packed red blood cells, 2,728 (41%) were FFP, 306 (5%) were platelets and 171 (3%) were cryoprecipitates. Waste plasma samples were stored from 3,813 (57%) transfusions for subsequent laboratory studies. Seventy-four (8%) patients developed ALI within 6 hours of transfusion (Figure 1). Interobserver agreement was moderately good (κ 0.6).

Table 1 describes clinical and transfusion characteristics of patients who developed ALI after transfusion and matched control subjects. Gastrointestinal bleeding was the most common reason for ICU admission (Table 1). Compared with patients who had no respiratory worsening after transfusion, patients who developed ALI were more likely to have sepsis, liver disease, and a history of chronic alcohol abuse (Table 1).

ALI case subjects were more likely to have received plasma-rich blood products (FFP or platelets). They were also more likely to have received blood products from female donors and larger volumes of plasma from female donors (Table 1). Five case subjects (7%) and four control subjects (5%) received massive transfusion (at least 10 units) during the exposure period. Donors to patients who developed ALI had a higher number of pregnancies and tested positive for anti-leukocyte antibodies (Tables 1 and 2) more often. There was no difference in IL-8 or storage age of red blood cell products between patients who developed ALI and matched control subjects. The concentration of LysoPC was significantly higher in blood products given to ALI case subjects than to control subjects (Table 2). At least one of the associated units was tested for anti-leukocyte antibodies for 82% of case subjects and 80% of control subjects. LysoPC and IL-8 were tested in at least one associated unit for 73% of case subjects and 78% of control subjects (Table 2).

Table 3 provides the unadjusted and adjusted ALI odds ratios for specific transfusion risk factors. When adjusted for baseline APACHE III scores, sepsis, and alcohol abuse in the conditional logistic regression analysis, several transfusion factors remained associated with the development of ALI (Table 3). The results were similar after additional adjustment for baseline liver disease (*see* Table E2 in the online supplement). The distribution of transfusion factors was similar in subgroups of patients who had significant additional ALI risk factors ("possible TRALI") and those who did not ("suspected" TRALI) (*see* Table E1).

Of 74 patients who developed ALI, 58 were treated with mechanical ventilation (48 invasive, 10 noninvasive) and 16 were treated with oxygen supplementation via face mask. The median duration of mechanical ventilation was 3.6 (1.6 to 7.1)

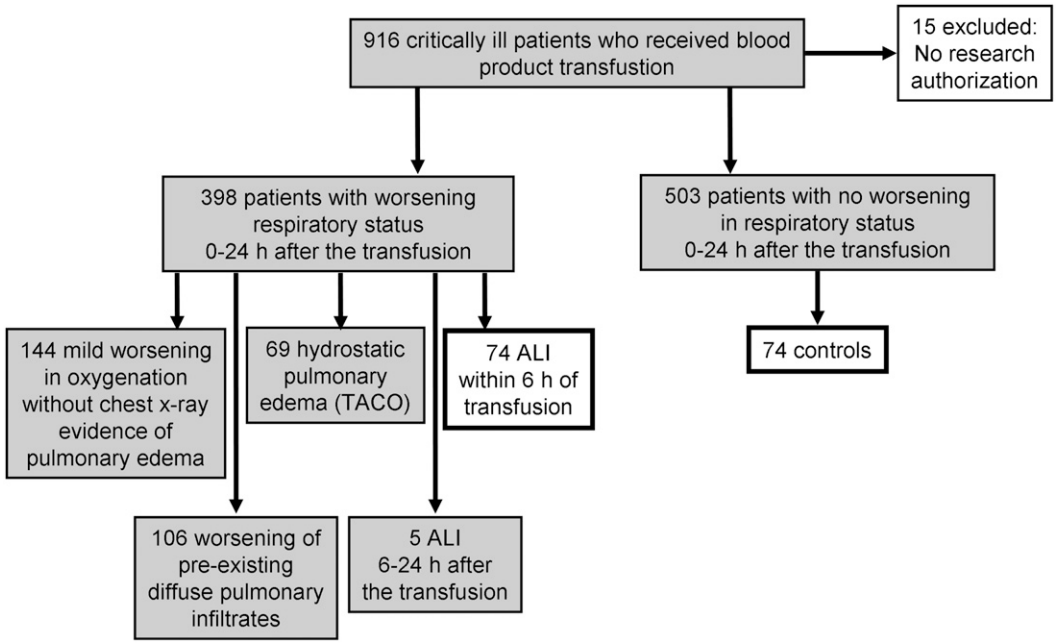

Figure 1. Outline of the study. ALI = acute lung injury; TACO = transfusion-associated circulatory overload.

days. Twenty-seven case subjects and 27 control subjects were mechanically ventilated at the time of transfusion. An additional 31 case subjects were started on mechanical ventilation (26 invasive, 5 noninvasive) because of the development of ALI. Hospital mortality was higher in patients who developed ALI (41%) than in matched control subjects (23%) ($P < 0.01$) (*see* also the online supplement).

DISCUSSION

The principal findings of our study are the following: (*1*) in a cohort of critically ill medical patients, the development of ALI shortly after transfusion was more common than usually appreciated and an order of magnitude more common than what is reported to blood banks; (*2*) both underlying patient characteristics ("first hit") and specific transfusion factors ("second hit") were associated with the development of ALI after blood transfusion; and (*3*) our results support both of the proposed TRALI mechanisms as well as the general multiple-hit model of TRALI and ALI.

To our knowledge, this is the first time the recommended standardized clinical definition (3, 4, 22) was implemented in a prospective study of TRALI. In critically ill medical patients admitted to a tertiary care medical center, clinically defined TRALI appears to be more common than previously thought, supporting the general notion that TRALI is grossly underrecognized and underreported (3–5). Rather than concentrating on clinically suspected TRALI reactions reported to blood banks, we prospectively examined consecutive transfused critically ill medical patients. Patients who developed ALI after transfusion often had other important ALI risk factors not related to the transfusion itself, for example, sepsis and a history of chronic alcohol abuse. The fact that potentially modifiable transfusion risk factors (such as donor sex, parity, and alloimmunization) are commonly associated with the development of ALI may have important implications in the prevention and treatment of ALI in critically ill medical patients. Observational data already suggest a decrease in incidence of ALI in patients treated according to a less liberal transfusion policy (32, 33). Moreover, preliminary data from the United Kingdom suggest a significant decrease in postoperative ALI associated with a decreased use of FFP from female donor plasma (34).

Our results to some extent support both of the main proposed mechanisms for TRALI (6, 7, 35, 36) as well as the overall multiple-hit paradigm in ALI development (13, 14). Both the presence of anti-leukocyte antibodies and the concentration of bioactive lipid factors in the donor product were higher in patients who developed ALI than in matched control subjects both with and without adjustment for baseline characteristics ("first hit"). Although the small sample size precludes wider inferences, a closer look at the distribution of antibodies and LysoPC across different blood products (*see* Tables E3 and E4) reveals a trend toward more antibody-positive units given to patients with ALI versus control subjects across each of the blood products (red blood cells, FFP, and platelets). On the other hand, a higher concentration of LysoPC seems merely to reflect the larger number of high plasma volume products (platelets and FFP) given to patients with ALI versus control subjects. Our study design did not allow us to determine the sensitivity and specificity of laboratory diagnosis of TRALI, or to assess the specific etiologic (as opposed to a biomarker) role of anti-leukocyte antibodies and LysoPC.

The association between infusion of plasma from female donors and the subsequent development of lung injury is an intriguing finding of our study and has important implications regarding the etiology and prevention of TRALI. Our findings support the results of the small randomized trial by Palfi and colleagues, in which plasma transfusion from multiparous female donors led to worsening in oxygenation and increased inflammatory response (35). The U.K. blood system has nearly eliminated female donors from the production of high plasma volume products (FFP) and the first reports suggest a significant decrease in TRALI reactions reported to the national transfusion surveillance system (SHOT [serious hazards of transfusion]) (37). The AABB TRALI Working Group has recommended similar guidelines for North American blood centers, with a plan to eliminate transfusion of high plasma volume products from donors at high risk of leukocyte alloimunization (including previously pregnant female donors) by November of

TABLE 1. DEMOGRAPHICS, ADMISSION DIAGNOSTIC GROUPS, PRETRANSFUSION ACUTE LUNG INJURY (ALI) RISK FACTORS, AND TRANSFUSION CHARACTERISTICS OF PATIENTS WHO DEVELOPED ALI AFTER TRANSFUSION AND OF MATCHED CONTROL SUBJECTS

	Patients with ALI (*n* = 74)	Matched Control Subjects (*n* = 74)	*P* Value
Age (yr), median (IQR)	64 (52 to 78)	61 (53 to 73)	
Female sex, n (%)	37 (50)	37 (50)	
ICU admission diagnosis, n (%)			
Gastrointestinal	28 (38)	28 (38)	
Respiratory	18 (24)	18 (24)	
Cardiovascular	17 (23)	17 (23)	
Genitourinary	6 (8)	5 (7)	
Hematology	4 (6)	5 (7)	
Metabolic	1 (1)	1 (1)	
Baseline APACHE III score	61 (44 to 75)	57 (45 to 81)	0.586
Sepsis, n (%)	27 (37)	16 (22)	0.016
Pneumonia, n (%)	5 (7)	7 (9)	0.683
Aspiration, n (%)	8 (11.4)	13 (18.6)	0.225
History of heavy alcohol use, n (%)	27 (36.5)	13 (17.6)	0.006
Liver disease, n (%)	20 (27.4)	11 (15.1)	0.039
Pancreatitis, n (%)	5 (6.8)	1 (1.4)	0.102
DIC, n (%)	10 (13.9)	5 (6.9)	0.132
Patients receiving RBC transfusion, n (%)	46 (62.2)	54 (73)	0.144
Patients receiving high plasma volume components (FFP or platelets), n (%)	44 (59.5)	27 (36.5)	0.006
Average RBC storage age, d (n = 35 matched pairs), median (IQR)	22.9 (17 to 31)	22.9 (15 to 30)	0.801
Number of associated units, median (IQR)*	3 (1 to 5)	2 (1 to 3)	0.063
RBCs	1 (0 to 2)	2 (0 to 2)	0.77
FFP	1 (0 to 4)	0 (0 to 1)	0.08
Platelets†	0 (0 to 0)	0 (0 to 0)	0.06
Number of units from female donors, median (IQR)	1 (1 to 2)	1 (0 to 2)	0.016
Number of pregnancies among donors (n = 68 matched pairs), median (IQR)	2 (0 to 5)	0 (0 to 3)	0.030
Amount of plasma (L), median (IQR)	0.27 (0.07 to 1.03)	0.07 (0.07 to 0.32)	0.047
Amount of plasma from female donors (L), median (IQR)	0.16 (0.04 to 0.5)	0.04 (0 to 0.1)	0.013
Amount of plasma (L) from female donors with at least one pregnancy (n = 68 matched pairs), median (IQR)	0.04 (0 to 0.25)	0 (0 to 0.07)	0.038

Definition of abbreviations: ALI = acute lung injury; APACHE = Acute Physiology and Chronic Health Evaluation; DIC = disseminated intravascular coagulation; FFP = fresh-frozen plasma; IQR = interquartile range; RBC = red blood cells.

* Units transfused within 0–6 hours of the development of ALI in case subjects and within the first 0–6 hours after the first transfusion in control subjects.

† Of 16 platelet units given to cases, 10 were apheresis platelets and 6 were pooled platelets; of 6 platelet units given to control subjects, 3 were apheresis platelets and 3 were pooled platelets.

2008 (28). Because this intervention may be associated with costs and/or shortage of blood products in some blood centers, further studies will need to assess whether the benefit of this intervention outweighs the costs.

Relatively high mortality of our suspected or possible TRALI cases is comparable to a report from Wallis and colleagues (38) and our retrospective study of the critically ill (19), but higher than in some other epidemiological studies (6). It is important

TABLE 2. SPECIFIC LABORATORY TEST RESULTS OF DONOR SAMPLES GIVEN TO PATIENTS WHO DEVELOPED ACUTE LUNG INJURY AFTER TRANSFUSION AND TO MATCHED CONTROL SUBJECTS

	No. of Matched Pairs	Patients with ALI	Matched Control Subjects	*P* Value
IL-8 (pg/dl), median (IQR)	47	2.5 (2.5 to 6.4)	2.5 (2.5 to 7.7)	0.448
LysoPC 16:0† (per 10-μmol/L increase), median (IQR)	47	46 (21 to 109)	25 (18 to 72)	0.004
LysoPC 18:0† (per 10-μmol/L increase), median (IQR)	47	15 (4.9 to 33)	7.25 (2.5 to 19)	0.005
Number of antibody$^+$ tests, median (IQR)*	52	1 (0 to 2)	0 (0 to 1)	0.014
Received any HLA class I$^+$ units, n (%)	52	21 (40.4)	12 (23.1)	0.072
Received any HLA class II$^+$ units, n (%)	52	17 (32.7)	10 (19.2)	0.108
Received any GIF$^+$ units, n (%)	52	13 (25)	4 (7.7)	0.020
Percentage of associated units tested for anti-leukocyte antibodies, median (IQR)		67% (24 to 100)	90% (33 to 100)	0.341
Percentage of associated units tested for IL-8 and LysoPC, median (IQR)		50% (0 to 100)	67% (33 to 100)	0.036

Definition of abbreviations: GIF = granulocyte immunofluorescence; HLA = human leukocyte antigen; IQR = interquartile range; LysoPC = lysophosphatidylcholine.

* If the associated unit tested positive for more than one antibody (e.g., both HLA class I and HLA class II), each of these was counted as a positive test.

† 16:0 and 18:0 refer to palmitic and stearic acid, respectively.

TABLE 3. TRANSFUSION-RELATED RISK FACTORS FOR ACUTE LUNG INJURY

	Unadjusted*		Adjusted†	
Variable	OR (*95% CI*)	*P* Value	OR (*95% CI*)	*P* Value
Any high plasma volume components (FFP or platelets)	2.55 (1.27–5.11)	0.009	2.78 (1.21–6.38)	0.016
Number of units	1.09 (0.99–1.20)	0.081	1.11 (0.99–1.25)	0.086
Number of units from female donors	1.30 (1.03–1.66)	0.029	1.51 (1.08–2.12)	0.016
Amount of plasma from male donors, L	1.55 (0.79–3.06)	0.202	1.60 (0.76–3.37)	0.215
Amount of plasma from female donors, L	3.23 (1.17–8.91)	0.024	5.09 (1.37–18.85)	0.015
Amount of plasma from female donors with at least one pregnancy, L	4.41 (1.00–19.55)	0.050	9.48 (1.38–65.35)	0.022
Number of pregnancies among donors	1.11 (1.00–1.22)	0.047	1.19 (1.05–1.34)	0.007
Number of HLA class I^+ units	1.81 (0.97–3.38)	0.061	1.70 (0.94–3.09)	0.098
Number of HLA class II^+ units	1.93 (0.88–4.28)	0.103	3.08 (1.15–8.25)	0.025
Number of GIF^+ units	4.19 (1.22–14.32)	0.023	4.85 (1.32–17.86)	0.018
Mean LysoPC 16:0** (per 10-mol/L increase)	1.16 (1.04–1.30)	0.011	1.16 (1.02–1.32)	0.022
Mean LysoPC 18:0** (per 10-mol/L increase)	1.58 (1.10–2.26)	0.013	1.61 (1.08–2.38)	0.018

Definition of abbreviations: CI = confidence interval; FFP = fresh-frozen plasma; LysoPC = lysophosphatidylcholine; OR = odds ratio.

For continuous variables, ORs were calculated per unit of measurement: for each additional unit transfused, for each additional liter of plasma (1 L of plasma corresponds to a usual dose of about 4 units of FFP), for each 10-μmol/L increase in LysoPC).

* Unadjusted for baseline APACHE III score, sepsis, and chronic alcohol abuse.

† Adjusted for baseline APACHE III score, sepsis, and chronic alcohol abuse.

** 16:0 and 18:0 refer to palmitic and stearic acid, respectively.

to emphasize the characteristics of the population studied: medical ICU patients in a tertiary care medical center, a group with an inherently high mortality rate. The observed mortality may or may not be attributable to TRALI as these critically ill patients had multiple additional risk factors for both ALI and poor outcome.

Our study design includes some limitations. The study was conducted in a single medical ICU in a tertiary care center and, although internal validity may be high, external validity is limited. In addition, the observational nature of the study does not allow independent estimation of the cause-and-effect relationship between the predictors and outcome. Residual confounding is always an issue in observational studies but is unlikely to fully explain our observed differences in donor product characteristics (anti-leukocyte antibodies from previously pregnant female donors and the concentration of LysoPC). Because there is no way that clinicians could have specifically ordered "male" or "low LysoPC" product in our institution, indication bias should be less of an issue. Although the trend toward higher amounts of total plasma could explain some of the observed differences it is again unlikely to explain the association between the donor sex and pregnancy and the development of ALI.

An important limitation of our study is that we did not test for corresponding leukocyte antigens and were not able to determine the specificities of donor antibodies. We did not perform additional testing or treatment of the sera (e.g., adsorption of HLA class I antibodies with platelets) to confirm whether our granulocyte immunofluorescence-positive samples were indeed directed against granulocyte-specific antigens. Because the fresh granulocyte panels used for the assay could not be selected to include all possible granulocyte antigens, the potential exists that antibodies directed toward uncommon granulocyte antigens would not be detected. Therefore, we could not distinguish between the etiologic, rather than a biomarker, role of specific antibodies. In a number of units given to both case and control subjects we were unable to extract an adequate sample for laboratory analysis. Although the proportion of units tested for anti-leukocyte antibodies was similar for case and control subjects, a somewhat higher proportion of control units was tested for IL-8 and LysoPC. This difference, if anything, would make it less likely that a significant difference would be detected by chance alone.

Although the differences in pretransfusion risk factors may indicate a limitation of our matching procedure, we were able to distinguish important, but not as well appreciated, patient risk factors, that is, chronic alcohol abuse. Moss and coworkers previously reported a strong independent association between alcohol use and the development of ALI (27). Laboratory experiments suggest the depletion of antioxidant glutathione stores associated with chronic alcohol abuse as the most plausible mechanistic explanation (39). Because neutrophil-mediated oxidative injury is the hallmark of TRALI (7, 8, 36, 40), it seems plausible that antioxidant depletion may be associated with greatly increased risk of this type of ALI.

In conclusion, by prospectively applying the standardized clinical definition, we have found a high incidence of suspected and possible TRALI among transfused critically ill medical patients. The association between specific donor and transfusion characteristics and subsequent development of ALI has important implications relative to both the etiology and prevention of this syndrome. Ongoing multicenter studies in transfused patients with and without underlying critical illness will allow us to further elucidate the incidence and mechanisms of TRALI and the effect of potential preventive strategies.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript

References

1. Popovsky MA, Abel MD, Moore SB. Transfusion-related acute lung injury associated with passive transfer of antileukocyte antibodies. *Am Rev Respir Dis* 1983;128:185–189.
2. Holness L, Knippen MA, Simmons L, Lachenbruch PA. Fatalities caused by TRALI. *Transfus Med Rev* 2004;18:184–188.
3. Toy P, Popovsky MA, Abraham E, Ambruso DR, Holness LG, Kopko PM, McFarland JG, Nathens AB, Silliman CC, Stroncek D. Transfusion-related acute lung injury: definition and review. *Crit Care Med* 2005; 33:721–726.
4. Kleinman S, Caulfield T, Chan P, Davenport R, McFarland J, McPhedran S, Meade M, Morrison D, Pinsent T, Robillard P, *et al.* Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel. *Transfusion* 2004;44:1774–1789.
5. Kopko PM, Marshall CS, MacKenzie MR, Holland PV, Popovsky MA. Transfusion-related acute lung injury: report of a clinical look-back investigation. *JAMA* 2002;287:1968–1971.
6. Popovsky MA, Moore SB. Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. *Transfusion* 1985;25:573–577.
7. Silliman CC, Paterson AJ, Dickey WO, Stroneck DF, Popovsky MA, Caldwell SA, Ambruso DR. The association of biologically active

lipids with the development of transfusion-related acute lung injury: a retrospective study. *Transfusion* 1997;37:719–726.
8. Sachs UJ, Hattar K, Weissmann N, Bohle RM, Weiss T, Sibelius U, Bux J. Antibody-induced neutrophil activation as a trigger for transfusion-related acute lung injury in an *ex vivo* rat lung model. *Blood* 2006; 107:1217–1219.
9. Silliman CC, Voelkel NF, Allard JD, Elzi DJ, Tuder RM, Johnson JL, Ambruso DR. Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. *J Clin Invest* 1998;101: 1458–1467.
10. Densmore T, Goodnough L, Ali S, Dynis M, Chaplin H. Prevalence of HLA sensitization in female apheresis donors. *Transfusion* 1999;39: 103–106.
11. Looney MR, Gropper MA, Matthay MA. Transfusion-related acute lung injury: a review. *Chest* 2004;126:249–258.
12. Boshkov L. Transfusion-related acute lung injury and the ICU. *Crit Care Clin* 2005;21:479–495.
13. Silliman CC, Ambruso DR, Boshkov LK. Transfusion-related acute lung injury (TRALI). *Blood* 2005;105:2266–2273.
14. Matthay MA, Zimmerman GA, Esmon C, Bhattacharya J, Coller B, Doerschuk CM, Floros J, Gimbrone MA Jr, Hoffman E, Hubmayr RD, *et al.* Future research directions in acute lung injury: summary of a National Heart, Lung, and Blood Institute working group. *Am J Respir Crit Care Med* 2003;167:1027–1035.
15. Hudson LD, Milberg JA, Anardi D, Maunder RJ. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;151:293–301.
16. Khan H, Belsher J, Yilmaz M, Afessa B, Moore SB, Hubmayr RD, Gajic O. Fresh frozen plasma and platelet transfusions are associated with development of acute lung injury in critically ill medical patients. *Chest* 2007;131:1308–1314.
17. Gong MN, Thompson BT, Williams P, Pothier L, Boyce PD, Christiani DC. Clinical predictors of and mortality in acute respiratory distress syndrome: potential role of red cell transfusion. *Crit Care Med* 2005; 33:1191–1198.
18. Gajic O, Rana R, Mendez JL, Rickman OB, Lymp JF, Hubmayr RD, Moore SB. Acute lung injury after blood transfusion in mechanically ventilated patients. *Transfusion* 2004;44:1468–1474.
19. Rana R, Fernandez-Perez ER, Khan SA, Rana S, Winters JL, Lesnick TG, Moore SB, Gajic O. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. *Transfusion* 2006;46:1478–1483.
20. Silliman CC, Boshkov LK, Mehdizadehkashi Z, Elzi DJ, Dickey WO, Podlosky L, Clarke G, Ambruso DR. Transfusion-related acute lung injury: epidemiology and a prospective analysis of etiologic factors. *Blood* 2003;101:454–462.
21. Gajic O, Rana R, Winters JL, Mendez JL, Rickman OB, Evenson LK, Malinchoc M, Yilmaz M, DeGoey SR, Rasmussen DL, *et al.* Transfusion related acute lung injury in the medical critically ill patients: nested case–control study [abstract]. *Am J Respir Crit Care Med* 2007;175:A965.
22. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American–European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;149:818–824.
23. 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–S113.
24. Knaus WA, Wagner DP, Draper EA, Zimmerman JE, Bergner M, Bastos PG, Sirio CA, Murphy DJ, Lotring T, Damiano A, *et al.* The APACHE III prognostic system: risk prediction of hospital mortality for critically ill hospitalized adults. *Chest* 1991;100:1619–1636.
25. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ; ACCP/SCCM Consensus Conference Committee; American College of Chest Physicians/Society of Critical Care Medicine. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 1992;101:1644–1655.
26. Bartlett JG, Dowell SF, Mandell LA, File TM Jr, Musher DM, Fine MJ; Infectious Diseases Society of America. Practice guidelines for the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2000;31:347–382.
27. Moss M, Bucher B, Moore FA, Moore EE, Parsons PE. The role of chronic alcohol abuse in the development of acute respiratory distress syndrome in adults. *JAMA* 1996;275:50–54.
28. AABB. Transfusion-related acute lung injury. Association bulletin #06–07. Bethesda, MD: AABB; 2006.
29. Pei R, Lee J, Chen T, Rojo S, Terasaki PI. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. *Hum Immunol* 1999;60:1293–1302.
30. Verheugt FW, von dem Borne AE, Décary F, Engelfriet CP. The detection of granulocyte alloantibodies with an indirect immunofluorescence test. *Br J Haematol* 1977;36:533–544.
31. Silliman CC, Clay KL, Thurman GW, Johnson CA, Ambruso DR. Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. *J Lab Clin Med* 1994;124:684–694.
32. Ciesla D, Moore E, Johnson J, Cothren C, Banerjee A, Burch J, Sauaia A. Decreased progression of postinjury lung dysfunction to the acute respiratory distress syndrome and multiple organ failure. *Surgery* 2006;140:640–648.
33. Yilmaz M, Afessa B, Keegan MT, Hubmayr RD, Gajic O. The effect of ventilation and transfusion protocols on prevention of acute lung injury in mechanically ventilated patients. *Crit Care Med* 2006;43(12 Suppl):A85.
34. Wright S, Athey S, Leaver A, Snowden C, Roberts D, Clarkson J, Chapman C, Wallis J. The effect of male-donor-only fresh frozen plasma on the incidence of acute lung injury following ruptured abdominal aortic aneurysm repair. *Crit Care* 2007;11:374.
35. Palfi M, Berg S, Ernerudh J, Berlin G. A randomized controlled trial of transfusion-related acute lung injury: is plasma from multiparous blood donors dangerous? *Transfusion* 2001;41:317–322.
36. Seeger W, Schneider U, Kreusler B, von Witzleben E, Walmrath D, Grimminger F, Neppert J. Reproduction of transfusion-related acute lung injury in an *ex vivo* lung model. *Blood* 1990;76:1438–1444.
37. Chapman CE, Williamson LM, Cohen HEA. The impact of using male only plasma on hemovigilance reports of transfusion-related acute lung injury (TRALI) in the UK [abstract]. *Vox Sang* 2006;91(Suppl 3):227.
38. Wallis JP, Lubenko A, Wells AW, Chapman CE. Single hospital experience of TRALI. *Transfusion* 2003;43:1053–1059.
39. Moss M, Guidot DM, Wong-Lambertina M, Ten Hoor T, Perez RL, Brown LA. The effects of chronic alcohol abuse on pulmonary glutathione homeostasis. *Am J Respir Crit Care Med* 2000;161:414–419.
40. Looney MR, Su X, Van Ziffle JA, Lowell CA, Matthay MA. Neutrophils and their Fc γ receptors are essential in a mouse model of transfusion-related acute lung injury. *J Clin Invest* 2006;116:1615–1623.