Transfusion-Related Acute Lung Injury: Current Concepts for the Clinician

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The leading cause of transfusion-related morbidity and mortality in the United States is transfusion-related acute lung injury (TRALI). Diagnostic criteria for TRALI have recently been developed and primarily consist of hypoxia and bilateral pulmonary edema occurring during or within 6 h of a transfusion in the absence of cardiac failure or intravascular volume overload. The primary differential diagnosis is transfusion-associated circulatory overload and differentiation can be difficult. Treatment is supportive with oxygen and mechanical ventilation. Diuresis is not indicated and the role of steroids is unproven. Patients typically recover within a few days. All types of blood products have been associated with TRALI, however, the plasma-rich components, such as fresh frozen plasma and apheresis platelets, have been most frequently implicated. The pathogenesis of TRALI is not completely understood. Leukocyte antibodies in donor plasma have been implicated in most cases with antibodies directed at human leukocyte antigen (HLA) class I, HLA class II or neutrophil-specific antigens, particularly HNA-3a. Activation of pulmonary endothelium is important in the development of TRALI and may account for most cases being observed in surgical or intensive care unit patients. Transfused leukoagglutinating antibodies bind to recipients’ neutrophils localized to pulmonary endothelium resulting in activation and release of oxidases and other damaging biologic response modifiers that cause capillary leak. In a minority of TRALI cases, no antibodies are identified and it is postulated that neutrophil priming factors in the transfused component can mediate TRALI in a patient with pulmonary endothelial activation, the so-called “two hit” mechanism. Recognition of the role of anti-leukocyte antibodies has led to new strategies to reduce the risk of TRALI. Female blood donors with a previous pregnancy frequently have HLA antibodies with an overall prevalence of 24% and increasing prevalence related to the number of previous pregnancies. Since HLA antibodies have been implicated in TRALI, blood centers have adopted policies to produce plasma components primarily from male donors. Strategies to reduce the risk from apheresis platelets are problematic and are likely to involve testing female apheresis platelet donors for HLA antibodies. Much more research is needed to understand the blood component and patient risk factors for TRALI so that novel strategies for treatment and additional measures to reduce the risk of TRALI can be developed.

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led the National Heart, Lung, and Blood Institute (NHBLI) to convene a working group of experts to identify research needs and provide a clinically useful definition. The group defined TRALI as “new acute lung injury (ALI) occurring during or within 6 h after a transfusion, with a clear temporal relationship to transfusion, in patients without or with risk factors for ALI other than transfusion.” Limitations to the proposed NHBLI working group definition are the exclusion of patients with preexisting ALI, excluding cases with onset more than 6 h after transfusion, lack of laboratory diagnostic criteria and a required severity of hypoxia that could miss subtle cases of TRALI. A subsequent Canadian Consensus conference in April 2004 further modified the NHBLI working group criteria for TRALI (Table 1). The modified criteria broadened the definition of hypoxia to include clinical evidence of hypoxia and created a category of “possible TRALI” to address cases in which patients have other risk factors for ALI such as sepsis, aspiration, near-drowning, disseminated intravascular coagulation, trauma, pneumonia, drug overdose, fracture, burns and cardiopulmonary bypass. It was acknowledged that subtle cases may not meet the Canadian Consensus definition, nor was there agreement on how to diagnose subtle cases. However, these criteria would allow for the great majority of clinically significant cases to be captured.

Clinical and Laboratory Features

TRALI has a clinical presentation mirroring acute respiratory distress syndrome occurring in the setting of transfusion. Patients present with respiratory distress (dyspnea), hypoxia, pulmonary edema on examination, and bilateral fluffy infiltrates on chest radiograph during or within 6 h of transfusion. The majority of cases occur during or within 1 to 2 h of transfusion. Signs and symptoms include tachypnea, frothy pulmonary secretions, hypotension (less commonly hypertension), fever, tachycardia and cyanosis. Auscultation of the lung fields reveals diffuse rales. The leaky capillary endothelium results in tracheal fluid that is characteristically exudative. Importantly, there is no evidence of circulatory overload with absence of jugular venous distension or a S3 gallop. Central venous pressure and pulmonary capillary wedge pressure is normal. B-natriuretic peptide may have some value in distinguishing transfusion-associated circulatory overload (TACO) from TRALI. TACO is suggested by an absolute B-natriuretic peptide level more than 100 pg/dl and a posttransfusion ratio to pretransfusion ratio more than 1.5. Laboratory findings in the acute setting are of limited value because they are only suggestive and not diagnostic of TRALI. Such findings include leucopenia, neutropenia, monocytopenia and hypocomplementemia. Laboratory tests which strongly support, but are not required for the clinical diagnosis of TRALI, include the demonstration of human leukocyte antigen (HLA) class I or class II or neutrophil-specific antibodies in donor plasma and the presence of the cognate (corresponding) antigen on recipient neutrophils (see section on Antibody-Mediated TRALI). Such testing typically takes days or weeks to perform and is not helpful clinically. Tests for lipid priming activity or neutrophil-activating factors in the plasma from the blood component are only available on a research basis.

The diagnosis of TRALI is based on clinical criteria derived from the NHBLI working group and Canadian Consensus conference guidelines. In practice it can be difficult to differentiate TRALI from TACO and it is possible that there may be both complications concurrently in a patient. One cannot make a diagnosis of TRALI with current criteria if a patient has ALI before the suspected transfusion. A diagnosis of possible TRALI can be made if there are preexisting risk factors for ALI other than transfusion. Undoubtedly, as experience in recognition and understanding of TRALI grows, the criteria will evolve with improved sensitivity and specificity for TRALI.

Management of TRALI

Treatment of TRALI is primarily supportive with supplemental oxygen and in most cases ventilatory support. In contrast to acute respiratory distress syndrome from other causes, patients typically recover quickly, with resolution of pulmonary infiltrates within 96 h of the transfusion. The mortality rate has been reported to be between 5% and 10%. Patients with TRALI are euvoletic or may be hypovolemic as a result of excessive fluid leakage into the lung. Whereas rapid volume reduction with diuresis is the treatment of choice for TACO, in patients with

Table 1. Canadian Consensus Conference Proposed Criteria for Transfusion-Related Acute Lung Injury (TRALI)

<table>
<thead>
<tr>
<th>Criteria for TRALI</th>
<th>ALI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lung injury (ALI)</td>
<td>Acute onset</td>
</tr>
<tr>
<td>Hypoxemia</td>
<td>In research setting</td>
</tr>
<tr>
<td></td>
<td>Ratio of PaO2/FIO2 ≤ 300 or</td>
</tr>
<tr>
<td></td>
<td>SpO2 &lt; 90% on room air</td>
</tr>
<tr>
<td>Nonresearch setting</td>
<td>Ratio of PaO2/FIO2 ≤ 300 or</td>
</tr>
<tr>
<td></td>
<td>SpO2 &lt; 90% on room air</td>
</tr>
<tr>
<td>Other clinical evidence of hypoxia</td>
<td></td>
</tr>
<tr>
<td>Bilateral infiltrates on frontal chest</td>
<td></td>
</tr>
<tr>
<td>radiograph</td>
<td></td>
</tr>
<tr>
<td>No evidence of left atrial hypertension</td>
<td></td>
</tr>
<tr>
<td>(i.e., circulatory overload)</td>
<td></td>
</tr>
<tr>
<td>No preexisting ALI before transfusion</td>
<td></td>
</tr>
<tr>
<td>During or within 6 h of transfusion</td>
<td></td>
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<tr>
<td>and No temporal relationship to an alternative risk factor for ALI</td>
<td></td>
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</tbody>
</table>

Criteria for possible TRALI

| ALI                                      |                                           |
| No preexisting ALI before transfusion   |                                           |
| During or within 6 h of transfusion     |                                           |
| A clear temporal relationship to an alternative risk factor for ALI |

TRALI, diuretics may cause hypovolemia. There are no data regarding the efficacy of steroids, so their role remains unclear.

### Incidence and Implicated Blood Components

TRALI has clearly emerged as the leading cause of transfusion-related mortality representing the leading cause of transfusion-related death reported to the FDA since 2003. In 2006, the most recent year of data, there were 35 reported deaths due to TRALI, more than all other causes of transfusion-related mortality combined. The true incidence of TRALI is unknown because a standardized definition has only recently been developed. Initial studies quoted a per component incidence of 1:5000 blood components with subsequent reports ranging from 1:432 whole blood platelets to 1:557,000 red cells. TRALI has been reported from all types of blood components including whole blood, red cells, apheresis platelets, whole blood platelets, fresh frozen plasma, cryoprecipitate, granulocytes, stem cell products and even IV immunoglobulin preparations. Most implicated blood products contain more than 50 mL of plasma. A recent report of a passive TRALI surveillance system in the American Red Cross from 2003 to 2005 used the Canadian Consensus conference as the primary diagnostic criteria. They reported a per component risk of fatality from probable TRALI as shown in Fig. 1. Fresh frozen plasma has also been implicated most frequently in TRALI cases and TRALI-related deaths reported to the FDA and United Kingdom. The only prospective cohort clinical surveillance study to use current diagnostic guidelines for TRALI reported an 8% incidence of TRALI in their intensive care unit population and that plasma and platelet transfusions were associated with greatest risk of TRALI. These data suggest that passive reporting systems greatly under-estimate the number of TRALI cases. However, both passive and prospective surveillance studies using current criteria for TRALI diagnosis suggest that the plasma-rich components, such as plasma and apheresis platelets have the highest per component risk.

### Pathophysiology of TRALI

The exact mechanism of TRALI is not fully understood. An immune antibody-mediated mechanism has been implicated in most cases of TRALI. In a minority of reported cases, however, an antibody was not identified and an alternative “two hit” immune mechanism has been postulated. Data from animal models of TRALI and more recent clinical data have suggested that both mechanisms occur and that TRALI may represent the final common pathway of neutrophil priming, activation, endothelial injury and capillary leak, which can be triggered by antibodies and/or other biologic response modifiers in patients with or without underlying risk factors. The pathogenesis of TRALI has been recently elegantly reviewed by Bux and Sachs (Fig. 2).

### Antibody-Mediated TRALI

The antibody-mediated mechanism postulates that passive transfer of leukoagglutinating antibodies via transfusion of plasma containing blood components results in binding to recipient neutrophils. Antibody-bound neutrophils are activated and sequestered in the lungs where complement activation and release of neutrophil bioactive products results in endothelial damage, capillary leak and ALI. In 65% to 90% of reported clinical cases of TRALI, leukocyte antibodies have been identified in the implicated donor. Antibodies which have been implicated in TRALI include donor HLA class I, HLA class II, and/or neutrophil-specific antibodies. In the prospective surveillance study by Gajic et al., the odds
Alloimmunization to neutrophil-specific antibodies is highly correlated with parity, with alloimmunization rates in women with zero, 1, 2, 3 or more antibodies being much less frequent. The prevalence of HLA class I and class II antigens has been recently studied in more than 5000 United States (US) female blood donors using sensitive flow cytometry-based multi-antigen Luminex testing. The prevalence of HLA class I and class II antibodies is highly correlated with parity, with alloimmunization rates in women with zero, 1, 2, 3 or more pregnancies of 1.7%, 11.2%, 22.3%, and 29.8%, respectively. Alloimmunization to neutrophil-specific antigens is much less frequent 0.1–1.8%. HLA class II antigens are also expressed on monocytes and may contribute to TRALI.

Figure 2. a–c, Pathophysiology of transfusion-related acute lung injury (TRALI). Under physiologic conditions, the granulocytes, which has an average size similar to, or larger than, the diameter of a lung capillary, has to squeeze itself through many of the lung capillaries during its passage through the lungs (a). Activated neutrophils are trapped in the pulmonary vasculature, a prerequisite for the initiation of TRALL Two models have been proposed. If transfused antibodies induce granulocyte agglutination, this leukoagglutinate will be trapped in the lung vessels, a model referred to as immune TRALI (b). In severely ill patients, the reactivity of the endothelium or the granulocyte may be enhanced due to the underlying disease; if biologically active lipids, which accumulate during storage of cellular blood components, are transfused, the trapped granulocyte is finally activated and induces TRALI a model referred to as nonimmune TRALI (c). (Reproduced with kind permission of Springer Science + Business Media.)

ratio for TRALI was increased in recipients of blood units that contained HLA class II and neutrophil antibodies and a trend for those containing HLA class I antibodies. Although a number of neutrophil-specific antibodies have been reported, the most common is directed at the 5b (HNA-3a) antigen. In a small percent of cases, the leukoagglutinating antibody appears to be from the recipient and is directed at the transfused neutrophils. Most of the donors implicated in TRALI have been multiparous women who became alloimmunized during pregnancy. The frequency of sensitization to HLA class I and class II antigens has been recently studied in more than 5000 United States (US) female blood donors using sensitive flow cytometry-based multi-antigen Luminex testing. The prevalence of HLA class I and class II antibodies is highly correlated with parity, with alloimmunization rates in women with zero, 1, 2, 3 or more pregnancies of 1.7%, 11.2%, 22.3%, and 29.8%, respectively. Alloimmunization to neutrophil-specific antigens is much less frequent 0.1–1.8%. HLA class II antigens are also expressed on monocytes and may contribute to TRALI.

The antibody-mediated model is supported by both animal models and clinical data. Seeger et al. developed a rabbit ex vivo lung model for TRALI. Lung injury was observed when isolated lungs were perfused with human HNA-3a(5b) positive neutrophils followed by anti-5b and a source of complement, but not in the absence of anti-5b, use of 5b negative neutrophils or with control rabbit plasma. Similar results were found in a recent in vivo study two models which demonstrated ALI and increased mortality when a monoclonal MHC-I antibody was infused into mice with the cognate antigen. The lung injury was dependent on neutrophil expression of Fcγ receptors and was prevented in mice depleted of neutrophils with anti-granulocyte antibodies. A recently published ex vivo rat lung model found that lung injury occurred in rats perfused with human HNA-2a positive neutrophils followed by administration of a mouse monoclonal neutrophil antibody with HNA-2a (CD-177) specificity independent of the presence of complement. When neutrophils with <30% expression of anti–HNA-2a were used, the injury was greatly attenuated. Anti–HNA-2a was able to mediate lung damage without endotoxin activation of the lungs when high HNA-2a expressing neutrophils were used but endotoxin costimulation greatly accelerated lung vascular permeability. An in vitro model by Silliman et al. using human pulmonary microvascular endothelial cells (HMVECs) and human HNA-3a antibodies found that these antibodies caused HNA-3a positive neutrophils to become primed and elicit a respiratory burst. However, they only mediated the damage of the HMVECs if there was previous activation of the HMVECs with endotoxin. This study implies that there must be some endothelial activation for leukocyte antibodies to mediate endothelial damage. The necessity of pulmonary endothelial activation for antibody-mediated TRALI remains controversial. Differences in the models of Sachs and Silliman et al. include human versus mouse anti-neutrophil antibodies, different neutrophil antibody specificity and HMVEC culture versus ex vivo rat lungs as target endothelial cells. Sachs postulated that recipient neutrophils require a threshold stimulation that can be accomplished by antibody alone in some circumstances, but is facilitated by previously activated endothelium. The development of TRALI in healthy volunteers supports this theory.

A large body of circumstantial clinical evidence links TRALI to leukocyte antibodies by virtue of their presence in the large majority of reported cases. Other supportive clinical data include a prospective, randomized, crossover study of 2 U of plasma from multiparous donors (≥3 live births) versus control plasma in 105 intensive care patients. This study found that patients who received study plasma had impaired lung function as evidenced by a decrease in PaO2/FiO2 ratio versus controls. As previously noted, a prospective surveillance study by Gajic et al. reported that recipients of units with leukocyte antibodies had a higher risk of developing TRALI. Dykes et al. reported TRALI in a single-lung transplant recipient.
after a red cell transfusion containing HLA-B44 antibodies. Lung injury was found only in the B44 positive transplanted lung but not in the B44 negative native lung. The impact of switching to male plasma in the UK was recently reported in a before and after study of 211 patients who underwent repair of a ruptured abdominal aortic aneurysm. They found a reduction in the incidence of ALI within 6 h of surgery from 36% to 21% (P = 0.04) and fewer patients were hypoxic (87% vs 62%, P < 0.01).39 Thus clinical data and animal data support a leukocyte antibody-mediated mechanism for TRALI.

“Two Hit” Mechanism for TRALI

Despite the strong experimental and clinical evidence supporting an antibody mechanism of TRALI, there are inconsistencies. First, antibodies have not been found in 15% or more of cases of TRALI.10 The use of more sensitive assays for HLA or neutrophil antibodies or identification of antibodies to other cell types, such as monocytes21 or lymphocytes,22 may explain some of these cases. Second, although HLA antibodies are commonly found in female donors, only a very small proportion of these donors are implicated in TRALI. Third, donors with known HLA antibodies transfused to patients with the cognate antigen caused lung injury in some patients but not others.20,41 Fourth, patients who have experienced TRALI reactions do not always have the cognate antigen to leukocyte antibodies found in the implicated donor.21 An antibody-independent mechanism for TRALI has been proposed to explain these discrepancies. A two hit model has been proposed by Silliman et al.35,42,43 which postulates that an initial insult to vascular endothelium results in endothelial activation, release of cytokines and expression of adhesion molecules. Cytokines attract and prime neutrophils which firmly adhere to the endothelium. Events which could cause the initial proinflammatory endothelial activation include severe infection, surgery, trauma or massive transfusion. A second hit activates sequestered adherent neutrophils to release oxidases and proteases which damage the endothelium causing capillary leak and ALI. The second hit may be mediated by transfusion of biologic response modifiers, such as leukocyte antibodies, lipid priming molecules,44,45 cytokines, CD40 ligand46 or endotoxin.34,35 Animal studies have provided support for this hypothesis. An ex vivo rat model demonstrated ALI when rats were infused with endotoxin (first hit) and then followed with plasma from stored, but not fresh, platelets45 or red cells.44 Silliman et al. showed that infusion of lipids (lyso-phosphatidylcholines) extracted from the plasma supernatant of stored, but not fresh, platelets also caused ALI, demonstrating lipid priming activity as an important causative agent in stored blood components. The recent studies, mentioned above, by Sachs et al.34 using an ex vivo rat model and Silliman et al., using HMVECs in vitro75 further support the two hit hypothesis. Clinical studies also provide support for a nonimmune mechanism. A nested case-controlled study of 46 TRALI cases due to whole blood platelet concentrates found the same incidence of leukocyte antibodies in control donor components versus those implicated in TRALI.47 Furthermore, components implicated in TRALI had greater plasma-priming activity compared with components not implicated in TRALI. A smaller retrospective study of 10 consecutive patients with TRALI found HLA or granulocyte antibodies in donors in only 50% of cases and a predisposing event in all 10 patients.48 In this study, greater lipid priming activity was found in the plasma of patients with TRALI compared with controls or the patient’s pretransfusion samples. One limitation of the two hit model is the appearance of TRALI in patients who were apparently healthy before transfusion, such as in the setting of coumadin reversal for elective surgery.36,41 It is possible that such patients have subclinical evidence, or unrecognized risk factors for endothelial activation. Alternatively, a transfused component may contain a sufficiently strong antibody or provide both the mediators of endothelial activation and the second hit bioactive molecules which activate neutrophils and trigger TRALI.

Prevention of TRALI

Recent advances in the understanding of TRALI have led to new risk reduction measures. Until recently, the primary measure to prevent TRALI has been the deferral of donors implicated in a TRALI case. The AABB 24th edition of Standards for Blood Banks and Transfusion Services requires that donors implicated in a TRALI case be evaluated for eligibility to donate.49 There is no consensus regarding donor investigation or management of donors implicated in a TRALI case. Blood centers typically consider the likelihood that a case is bona fide TRALI, the number and types of donors involved and antibody specificity.50 Multiparous female donors are considered to be the highest risk donors. Donors found to have leukocyte antibodies corresponding to an antigen on the patient leukocytes or exhibiting a positive crossmatch between donor serum and patient leukocytes are deferred indefinitely or at least from donating plasma containing blood components.

Component factors have also been considered to reduce the risk of TRALI. The use of fresher platelets or red cells with reduced lipid priming activity1 or washed components has also been considered but is not currently recommended. A strategy that has been widely adopted is to use plasma exclusively or primarily from male donors and divert plasma from female donors to recovered plasma, which is only used for fractionation and production of plasma derivatives. The UK adopted this strategy in October 2003 with full implementation in January 2004. The UK Serious Hazards of Transfusion data showed that
TRALI cases associated with plasma-rich components decreased from 16 cases in 2003 to 3 cases in 2005. Importantly, which included minimizing preparation of plasma-diversion of female plasma could substantially reduce but are expected to be soon. May be another practical way to reduce the plasma components and replacement with platelet additive solutions. Lastly, removal of plasma from platelet components. It is feasible that such a loss could be replaced of approximately 6% of apheresis platelet donation was estimated to result in the loss plasma units in the US. Apheresis platelets contain a similar amount of plasma to plasma units; however, a similar strategy for platelets would be very difficult to implement due to supply limitations if female apheresis donors were not used. An alternative strategy would be to divert only those apheresis donors with a history of pregnancy or transfusion or to test female donors for leukocyte antibodies. An NHLBI funded Leukocyte Antibody Prevalence Study was recently completed by the REDSII research network. The study showed that transfusion had a minimal impact on HLA alloimmunization and that women who did not report a history of pregnancy had a prevalence of HLA antibodies similar to men. Among women who reported a history of pregnancy, 24% had class I and/or class II HLA antibodies. Diverting such donors from apheresis platelet donation was estimated to result in the loss of approximately 6% of apheresis platelet components. It is feasible that such a loss could be replaced with additional donors or use of whole blood platelets. Lastly, removal of plasma from platelet components and replacement with platelet additive solutions may be another practical way to reduce the plasma content and risk of TRALI from platelets. Platelet additive solutions are not currently licensed in the US but are expected to be soon.

Summary
TRALI has emerged as the leading cause of transfusion-related morbidity and mortality in the US. TRALI is a clinical diagnosis and is difficult to distinguish from TACO. Recently developed consensus clinical definitions will help provide consistency in identifying TRALI cases, facilitate establishing the true incidence of TRALI, and provide a framework from which laboratory and clinical studies can be performed. Such data will contribute to the development of novel strategies for treatment and additional measures to reduce the risk of TRALI.

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