# R E V I E W

# Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel

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Transfusion-related acute lung injury (TRALI) is an uncommon clinical complication of allogeneic blood transfusion. Despite its increasing recognition over the past 5 years, much about TRALI remains poorly understood and controversial. Outstanding issues include the lack of a universally accepted case definition, uncertainty about incidence and pathogenesis, and disagreement about both how to manage

**ABBREVIATIONS:** AECC = American-European Consensus Conference; ALI = acute lung injury; HMVEC(s) = human pulmonary microvascular endothelial cell(s); HNA(s) = human neutrophil antigen(s); LPS = lipopolysaccharide; lyso-PC(s) = lysophosphatidylcholine(s); SHOT = Serious Hazard of Transfusion.

donors who are associated with TRALI events and how to reduce the risk of TRALI for recipients of blood products.

To address these issues, a Consensus Conference was convened in Toronto, Canada, on April 1 and 2, 2004, entitled "Towards an Understanding of TRALI." The conference was sponsored by Canadian Blood Services and Héma-Québec, with support from the International Society of Blood Transfusion's Biomedical Excellence for Safer Transfusion (BEST) subcommittee. The format of the conference was based on that used by the National Institutes of Health and consisted of numerous expert presentations covering issues relevant to the topic, approximately 240 international attendees from a variety of backgrounds, and a consensus panel of 11 members covering a wide range of medical and other disciplines (e.g., transfusion medicine, epidemiology, immunology, anesthesiology, critical care medicine, and ethics as well as a regular blood donor and a chronic transfusion recipient). The Consensus Panel members, having first reviewed summaries of the TRALI literature and Consensus Panel procedures, convened immediately before the conference to clarify objectives, principles, and roles. Based on six questions posed by the Conference Steering Committee, the Consensus Panel mandate was to develop recommendations that could be applied both in Canada and internationally.

This Consensus Panel report is based on the information presented to the panelists during the conference, a review of background literature and continued postconference discussion. Given the absence of systematic reviews and randomized trials in this field, the Consensus Panel recommendations are not graded according to current evidence-based standards. In addition, no specific evidence was presented to the Consensus Panel regarding TRALI in neonates. Although TRALI among neonates is not excluded from these recommendations, we acknowledge the absence of data related to this specific population. This statement addresses the six specific questions posed by the conference steering committee. A publication of the full conference proceedings appears elsewhere.[1]

Question:

**A variety of complications can be associated with transfusion. How should TRALI be defined and what processes should be implemented to develop objective criteria for use in the classification of TRALI reactions?**

An appropriate definition of TRALI could vary according to the setting and purpose. Patient care clinicians need an inclusive definition that will allow for a timely diagnosis in the face of patient comorbidities and the limitations of available testing. For investigators evaluating interventions for TRALI prevention or treatment, a firm diagnosis based on objective criteria is highly desirable. This definition should necessarily be more exclusive. For surveillance initiatives and epidemiologic studies, an exclusive definition would underestimate incidence rates whereas an overinclusive definition could overestimate incidence and could also overwhelm efforts to evaluate implicated donors, recipients, and transfused products.

Defining TRALI requires a balance of precision and pragmatism. In any setting, TRALI cases might be classified as "mild," "moderate," or "severe" depending on immediacy of onset, duration, and degree of patient disability. Classification along a spectrum of certainty could lead to definitions of "possible," "probable," or "definite" TRALI. Uncertainty will inevitably arise from the confounding effects of underlying respiratory or cardiac disease (e.g., congestive heart failure), coexisting risk factors for acute lung injury (ALI; e.g., sepsis or shock), and other pulmonary complications of transfusion (e.g., volume overload, allergic reaction).

## FRAMEWORK

In defining TRALI, the Consensus Panel decided on some guiding principles. We sought portability, aiming to address the needs and exigencies of all settings. We strived for international acceptance, building on features of TRALI where most experts can agree and incorporating central features of definitions in current use. We also sought for simplicity, avoiding distinct definitions for different subgroups and clinical settings.

TRALI is one of many subsets of ALI that share a common spectrum of clinical, physiologic, and radiologic abnormalities. The American-European Consensus Conference (AECC) definition of ALI, first described in 1992, is widely employed by the international pulmonary and critical care community.[2] We adapted a previous recommendation by a US National Heart, Lung, and Blood Institute Working Group on TRALI that based its TRALI definition (presented as part of this conference program) on the AECC ALI definition.[1]

## DEFINITION

The Consensus Panel recommends that TRALI be defined as a new episode of ALI that occurs during or within 6 hours of a completed transfusion, which is not temporally related to a competing etiology for ALI (Table 1). The diagnosis of TRALI is a clinical and radiographic diagnosis and is not dependent on the results of laboratory tests or any proposed pathophysiologic mechanisms. TRALI should currently be considered a clinical syndrome rather than a disease with a single etiology.

Consistent with the AECC definition for ALI, we have defined TRALI by its timing, presence of hypoxemia, and chest radiograph abnormalities and the absence of evidence of circulatory overload, caused either by transfusion or by preexisting cardiac conditions. Consistent with pulmonary edema, chest radiograph abnormalities should show bilateral infiltrates that may be patchy, diffuse, homogeneous, or asymmetric and suggestive of alveolar or interstitial disease. Clinical evidence of circulatory overload is defined as a pulmonary capillary wedge pressure of 18 mmHg or greater, when a pulmonary arterial catheter is present. Otherwise, in many situations where this measurement is unavailable, clinicians must draw on other signs (jugular venous pulsations, breath sounds, S3 gallop), symptoms (orthostatic dyspnea), and other data (central venous pressure), to assist in their assessment of circulatory overload. All of this is concordant with the AECC definition for ALI.[2]

The ALI criteria for the definition of hypoxemia have been expanded in the Consensus Panel definition of TRALI. For clinical investigations we recommend defining hypoxemia as a ratio of the partial pressure of oxygen to the fractional inspired oxygen concentration ($PaO_2/FiO_2$) of less than 300 mmHg[2] or as an oxygen saturation measured by pulse oximetry of less than 90 percent when a

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**TABLE 1. Recommended criteria for TRALI and possible TRALI**

1. TRALI criteria
   a. ALI
      i. Acute onset
      ii. Hypoxemia
         Research setting:
            $PaO_2/FiO_2 \leq 300$,
            or $SpO_2 < 90\%$ on room air
         Nonresearch setting:
            $PaO_2/FiO_2 \leq 300$
            or $SpO_2 < 90\%$ on room air
            or other clinical evidence of hypoxemia
      iii. Bilateral infiltrates on frontal chest radiograph
      iv. No evidence of left atrial hypertension (i.e., circulatory overload)
   b. No preexisting ALI before transfusion
   c. During or within 6 hr of transfusion
   d. No temporal relationship to an alternative risk factor for ALI
2. Possible TRALI
   a. ALI
   b. No preexisting ALI before transfusion
   c. During or within 6 hr of transfusion
   d. A clear temporal relationship to an alternative risk factor for ALI

| TABLE 2. Risk factors for ALI* | |
|---|---|
| Direct lung injury | Indirect lung injury |
| Aspiration | Severe sepsis |
| Pneumonia | Shock |
| Toxic inhalation | Multiple trauma |
| Lung contusion | Burn injury |
| Near drowning | Acute pancreatitis |
| | Cardiopulmonary bypass |
| | Drug overdose |

* The incidence of ALI varies considerably among these conditions and may be as high as 40 percent for intensive care unit-related cases of septic shock and aspiration or as low as 2 percent for cases of cardiopulmonary bypass and intensive care unit-related drug overdose. The incidence and mortality of ALI in association with various defined risk factors has been summarized recently.[3]

| TABLE 3. Clinical symptoms and/or events observed in some TRALI cases |
|---|
| Dyspnea |
| Fever |
| Hypotension |
| Tachypnea |
| Tachycardia |
| Frothy endotracheal aspirate |
| Mechanical ventilation required to support oxygenation |
| Onset within 2 hr of transfusion |

patient is breathing room air. For the purpose of patient care and surveillance studies, however, when pulse oximetry measurements on room air may not be available, other clinical evidence of hypoxemia may suffice.

There are numerous clinical conditions in which ALI is known to occur independent of transfusion. (Table 2).[3] When ALI is *temporally* related to both transfusion and at least one other risk factor, the Consensus Panel recommends the use of the term "possible TRALI" rather than TRALI. The reasons for distinguishing TRALI from possible TRALI (i.e., those cases without another temporally related ALI risk factor from those cases with one) are multiple and include the uncertainty of the relationship of the ALI to the transfusion event in possible TRALI, the need to categorize such cases separately in surveillance systems to permit accurate comparisons across systems, the ability to selectively target research protocols to TRALI or possible TRALI cases, and the possible adoption of differing approaches to donor investigation in TRALI versus possible TRALI cases.

Importantly, the ALI risk factor of massive transfusion is not considered to be a predisposing condition that would require classification into possible TRALI and is not listed in Table 2. Provided that the onset of the clinical syndrome occurred within 6 hours of the last blood component transfused, a massively transfused patient with ALI symptoms would be considered a TRALI case, unless the patient had one or more conditions listed in Table 2.

The distinction between TRALI and possible TRALI is a subjective one, requiring a clinical assessment of the possible influence of the alternate ALI risk factor. Because of the subjectivity of such an assessment, the Consensus Panel recognizes the potential for a given case to be classified differently by different institutions and researchers. The Panel therefore urges that clear documentation concerning the time course of symptom evolution be provided for those cases in which there is an alternate ALI risk factor so that independent reviewers can also assess the

case. Factors that may provide suggestive evidence about whether a possible TRALI case was indeed caused by transfusion include the clinical course (TRALI is usually less severe and resolves more quickly than ALI owing to some other causes) and laboratory data that may be obtained as part of a TRALI case workup (see below). Until further knowledge is gained, however, the Panel suggests that the term possible TRALI for such cases be maintained for surveillance purposes. Detailed findings should be reported at scientific conferences and in the literature so that further scientific debate can ensue about when a case can be moved from a designation of possible TRALI to TRALI.

Table 3 outlines additional clinical signs and symptoms that have been frequently observed in TRALI cases but which lack appropriate specificity for inclusion in the definition of TRALI. These include dyspnea, tachycardia, hypotension unresponsive to fluid administration, and fever. Features of TRALI that are less clearly documented include hypertension, leukopenia, and hypocomplementemia.

## STRENGTHS AND LIMITATIONS

This definition for TRALI has a number of strengths. Based on an internationally accepted definition for ALI, and similar to other definitions of TRALI, this definition addresses the needs of varied settings and is relatively simple to employ. Moreover, the Consensus Panel deliberately avoided a classification scheme based on the pathophysiologic mechanisms of TRALI. Possible mild cases of TRALI are excluded (i.e., cases with some pulmonary symptoms that are not severe enough to meet the definition of TRALI) because no clear definition of such cases is yet available and because their inclusion would complicate tracking and comparison of TRALI cases in and between surveillance systems.

Under the proposed definition, cases in which circulatory overload is present are excluded as TRALI cases. The Panel recognizes that TRALI and circulatory overload can coexist, however, as has been well documented for ALI attributed to other causes.[4] It could be argued that such cases should be classified as possible TRALI so that they

are captured by surveillance systems and reported to blood centers for further workup. Although the Panel considered this possibility, it decided to exclude such cases from the possible TRALI category to be consistent with the 1994 AECC Consensus definition of ALI. Moreover, the Panel recognized that when TRALI coexists with circulatory overload, the latter, more transient condition might rapidly resolve with appropriate therapy (e.g., diuresis). At that time, if the TRALI persists, a diagnosis of TRALI may be made in hindsight and appropriately investigated. Of course, mild and self-limited TRALI cases in the setting of circulatory overload will be missed with this approach.

This definition also excludes a diagnosis of TRALI in a patient with preexisting ALI who subsequently receives a transfusion and whose ALI then worsens. Such cases are excluded because of the absence of a method to establish a causative link between the patient's clinical course and the transfusion.

The six-hour time limit for symptoms occurring following transfusion is consistent with most definitions that are currently in use. Moreover, data presented at this conference from the Serious Hazard of Transfusion (SHOT) surveillance system in the UK indicate that such a time limit will capture the large majority of TRALI cases. By use of a 24-hour posttransfusion time frame, SHOT reported that only 9 percent of suspected TRALI cases had symptoms that began more than 6 hours after transfusion.[1]

Finally, this definition of TRALI carries those limitations inherent to the AECC definition of ALI, most notably the subjectivity of volume status assessments and chest radiograph findings,[4,5] and the influence of positive end-expiratory pressure on measurements of $PaO_2$-to-$FiO_2$ ratio.

## FURTHER DEVELOPMENT

It is hoped that this proposed definition can be used as a starting point to arrive at an international consensus definition of TRALI and possible TRALI. Efforts are under way in this regard. These definitions should be expected to evolve as additional data are accumulated from improved TRALI surveillance, epidemiologic studies, systematic pathologic evaluations, and research into pathogenesis.

Question:

**The magnitude of the risk of TRALI is unknown at this time. What processes should be implemented to better define the magnitude of the TRALI risk?**

## INCIDENCE OF TRALI

The Panel agreed that the incidence of TRALI has not been well established. As seen in Table 4, incidence rates in the literature and in presentations made at the Consensus Conference vary widely[1] ranging from 1 in 432[6] to 1 in 88,000[7] per unit of platelets (PLTs), 1 in 8,000[8] to 1 in

**TABLE 4. Risk of TRALI per blood component transfused**

| Author (country) | Study period | Type of study | FFP | Whole blood-PLTs | Apheresis PLTs | Cryo-precipitate | RBCs | All |
|---|---|---|---|---|---|---|---|---|
| Silliman[6] (Canadian patients) | 1991-1995 | Enhanced surveillance, hospital | 1:19,411 | 1:432 | 1:1,224 | | 1:4,410 | 1:1,120 |
| Popovsky[9] (US) | Mid-1980s | Enhanced surveillance, hospital | | | | | | ~1:5,000 |
| Kopko[10] (USA) | 2001-2003 | Lookback investigation, blood center, hospitals | | | | | | ~1:25,000 |
| Quebec Hemovigilance (Canada)[1] | 2000-2003 | Passive surveillance, regional | 1:61,006 | 1:9,306 | see note | 1:25,073 | 1:58,279 | |
| SHOT[7] (UK) | 1996-2002 | Passive surveillance, national | 1:74,000 | see note | see note | 1:500,000 | 1:557,000 | |
| Wallis[8] (UK) | 1991-2002 | Case reports, hospital | 1:7,896 | | | | | |
| Silliman[12] (US) | Early 1990s | Retrospective study, hospital | | | | | | 1:2,000 |

Note: This report did not distinguish between whole blood PLTs and apheresis PLTs. The risk for PLTs was given as 1:88,000.

74,000[7] per unit of PLTs, and 1 in 4,000[6] to 1 in 557,000[7] per unit of red blood cells (RBCs).

The rates presented in Table 4 must be regarded with caution for three major reasons. First, the definition used for TRALI differed between studies. Some studies required that antibodies to human neutrophil antigen (anti-HNA) or anti-HLA antibodies be identified in the donor and that a positive cross-match with the recipient be demonstrated,[9] whereas others used only clinical criteria.[8] Although most studies required signs and symptoms to appear within 6 hours of transfusion,[9] one study chose 4 hours[8] and another chose 24 hours.[7] Second, the methods of surveillance differed; studies that involved active case investigation[6,9] reported higher rates than passive surveillance systems.[7] Third, the method of tabulating the denominator data of products transfused also varied across studies. Some studies used the number of products issued by blood centers,[7,10] whereas others used either a rough estimate[6,9] or the precise numbers of products transfused in the institutions where the study took place. (P. Robillard, personal communication).

Data on the severity and outcomes of TRALI are also limited. A mortality rate of 5 to 10 percent is commonly quoted, based primarily on the results of a single study.[9] Higher and lower rates have also been reported, however.

# METHODS TO IMPROVE KNOWLEDGE ON TRALI INCIDENCE

Accurate determination of incidence from various types of blood components is important because component-specific TRALI incidence may influence the decision to adopt certain preventative measures. In the future, two methods could be used to obtain data on TRALI incidence: surveillance systems and incidence studies.

To improve our knowledge of the incidence of TRALI, some prerequisites must be met. First, a standard definition of TRALI is needed so that comparison of cases across studies is valid. Because of the potential for factors other than transfusion to cause ALI in patients with severe clinical illness, the Consensus Panel encourages the classification of reactions into TRALI and possible TRALI and the recording of such cases independently in surveillance systems. Second, good denominator data is needed to calculate incidence rates. Theoretically, rates can be calculated based either on the number of recipients or on the number of products transfused. The Panel believes that both approaches to calculating TRALI incidence are useful and encourage investigators to report TRALI incidence in both ways when possible.

In the routine operational setting, it is usually easier to obtain data on number of products transfused compared to number of recipients transfused. If a product-based denominator is used, it should be based on the number of products by component type transfused (not

issued) in a given period of time in a given setting where the TRALI cases are studied (hospital, hospital network, province or state, country). The use of surrogate measurements (e.g., products issued) might lead to false international comparisons given that the transfusion practice and the issuance of blood vary substantially between countries. The use of products transfused, however, poses a classification problem in patients receiving blood components in that it may not always be possible to determine which type of blood product caused the TRALI episode. If recipient-based denominator data are collected, these data will be maximally useful if patients can be tabulated by the type of blood product received (e.g., RBCs vs. PLTs vs. FFP). Because patients often receive more than one type of blood product, however, it may be difficult to get accurate numbers of recipients in each product category.

## General surveillance systems

There are several surveillance systems for adverse transfusion reactions in existence throughout the world.[11] Interesting data on TRALI have been gathered through one of these systems, the SHOT system in the UK. Such systems rely on passive reporting of adverse transfusion reactions and are therefore subject to underreporting. With respect to TRALI, reasons for underreporting include the underrecognition of the syndrome by those who transfuse the products as well as the lack of reporting of these clinical events (and other adverse transfusion reactions) to the transfusion service (and subsequently to the blood center) for appropriate investigation.

The Consensus Panel recommends that the TRALI definition provided in this statement be adopted by existing surveillance systems such as the Transfusion-Transmitted Injury Surveillance System in Canada. The TRALI definition should be given to all clinicians and nurses who transfuse patients. Strategies for the appropriate dissemination of information on TRALI, such as active in-service training for nurses and residents and publications in prominent medical journals, should be adopted so that the syndrome is better recognized and reported. In addition, transfusion service personnel should work with nurses, anesthesiologists, and other physicians to heighten awareness of TRALI at their institutions and to improve surveillance.

## Reporting of TRALI cases

Standardized reporting mechanisms should be adopted within countries (and ideally internationally) to ensure comparability of data. Denominator data on products transfused should be collected by all surveillance systems.

Appropriate infrastructure should be provided to surveillance systems to ensure that training is provided and that adverse transfusion reactions, including TRALI, are appropriately investigated and reported. Some institu-

tions have transfusion safety officers to accomplish those tasks.

## Enhanced surveillance strategies

In some institutions with high transfusion volume, TRALI-specific enhanced surveillance strategies in designated services (like intensive care units) could be implemented. This would require special training of the personnel working in that unit to recognize, report, and investigate all suspected TRALI and possible TRALI cases. Ideally funds should be made available to encourage the formation of networks that would adopt such strategies. This mechanism would allow more precise estimates of the incidence of TRALI.

These sentinel sites could also provide valuable information on the extent of underreporting of TRALI in general surveillance systems. Another strategy to estimate underreporting would be to conduct lookback investigations on recipients of donors implicated in a TRALI case. This strategy is time-consuming and would best be considered a research activity.

## TRALI incidence studies

The best method to determine TRALI incidence accurately would be to closely monitor all transfusions for the appearance of clinical signs and symptoms related to TRALI and to ensure that appropriate recipient investigations (e.g., chest X-rays, hypoxemia measurements) are conducted to confirm the diagnosis. Given that TRALI is of low frequency, this is clearly a research strategy that would have to be multicenter in nature and would require substantial funding.

Question:

**What are the potential pathophysiologic mechanisms leading to TRALI and what research questions should be explored to investigate the mechanism(s) leading to TRALI?**

There are two proposed pathophysiologic mechanisms for TRALI: the antibody hypothesis and the neutrophil priming hypothesis.[10,12,13] Clinical and experimental observations support each of these mechanisms. In addition, several more speculative mechanisms have been proposed, including direct injury to pulmonary endothelium, immune complex formation with complement activation, and cytokine network activation. All proposed mechanisms lead to a final common pathway of increased pulmonary capillary permeability, resulting in pulmonary edema.[13]

The antibody hypothesis states that an antigen–antibody reaction triggers a series of events leading to TRALI. Most often, the causative blood component contains antibodies against recipient white blood cell (WBC) antigens. More rarely (e.g., in approx. 10 percent of those cases that

occur through the antigen–antibody mechanism), the antibody is present in the recipient and reacts with antigens on transfused donor WBCs. Antibodies may be HLA Class I or HLA Class II or directed against HNAs. It is possible that transfused HLA antibodies may directly activate or injure pulmonary endothelial cells. In experimental animal models, however, transfused antibodies work through a different mechanism, by binding to circulating WBCs, particularly neutrophils, causing cellular activation. Activated neutrophils, and possibly other WBCs, lodge in pulmonary capillaries either through cellular adhesive mechanisms or by physical trapping of WBC agglutinates. Such activated neutrophils release vasoactive substances, such as leukotrienes, or cytotoxic substances such as reactive oxygen metabolites. These mediators cause pulmonary endothelial leakage or damage with consequent pulmonary edema.

Evidence supporting the antibody hypothesis comes from many TRALI cases in which HLA or HNA antibodies have been demonstrated either in a transfused blood component or in the serum of an implicated blood donor. In many, but not all, such cases (approx. 40 in the literature), the corresponding antigen has been demonstrated in the recipient (or a positive cross-match between donor serum or plasma and recipient neutrophils has been demonstrated).[1,10] In two lookback investigations, previous donations from one donor with HLA and one donor with HNA antibodies whose blood components were found to have caused TRALI in an index recipient were found to have caused TRALI in previous recipients;[14,15] however, another lookback investigation reported entirely negative results in six investigated donors.[16] Although many HLA Class I and Class II specificities have been associated with TRALI, it is not clear from the present data whether there are particular HLA specificities that are more likely to cause TRALI. Case reports have associated each of the five currently identified HNA specificities with TRALI.[17] Anti-HNA-3a (formerly called anti-granulocyte 5b) has been implicated in several severe cases of TRALI, including several fatalities, indicating that this specificity may be particularly pathogenic for TRALI.[13,18]

One randomized controlled clinical trial provides some limited evidence in support of the antibody hypothesis.[19] Subjects (n = 105) received 1 unit of multiparous donor plasma (history of three or more pregnancies) and 1 unit of control plasma in random order separated by at least a 4-hour interval. Notably, many patients had a risk factor for ALI including pneumonia, sepsis, multiple trauma, and pancreatitis. There was a small but significant decrease in $PaO_2$-to-$FiO_2$ ratio associated with transfusion of multiparous plasma (before, $253.5 \pm 96.0$ mmHg; after, $233.3 \pm 98.3$ mmHg) that was not observed with control plasma. Five patients had clinical reactions, one of which was evidently TRALI. The implicated unit in this case was from a multiparous donor and contained immunoglobu-

lin G (IgG) neutrophil antibodies; however, a cross-match with the recipient's neutrophils was negative. This study suggests that transfusion of plasma from multiparous donors (presumably containing WBC antibodies) may have a mild deleterious effect on pulmonary function in the critically ill patient. Whether this effect is due to the transfusion of antibodies and whether such plasma carries an increased risk of causing clinical TRALI remains to be proven in larger prospective studies.

Experimental evidence in support of the antibody hypothesis comes from several animal models.[13] In a mouse model, BALB/c mice (H-2 $K^dD^d$) were injected intravenously with monoclonal anti-H-2 $K^dD^d$.[20] This resulted in rapid hypothermia, increase in lung water, and hemoconcentration compared to animals injected with nonspecific control antibody or H-2 $K^kD^k$ animals injected with anti-H-2 $K^dD^d$. Whole-animal spirometry indicated that a pulmonary response similar to dyspnea accompanied this reaction. Lung histology showed pulmonary edema and intravascular neutrophil accumulation, similar to descriptions of TRALI in autopsy cases. Depletion of neutrophils substantially reduced reactions caused by antibody injection, as did inhibition of the neutrophil IgG receptor FcγRIII.

A second experimental model supporting the antibody hypothesis is an ex vivo model.[21] Rat lungs were ventilated, perfused with physiologic buffer, and excised. In this method, ventilation and perfusion pressures were controlled. Human neutrophils were introduced into the perfusion circuit and subsequently monoclonal anti-HNA-2a or control antibody was added to the perfusate. Capillary permeability and lung weight increased after addition of neutrophils (>70% HNA-2a-positive) and anti-HNA-2a, but not with control antibody. The response was blunted when less than 30 percent HNA-2a-positive neutrophils were used. The onset of these events was accelerated by the addition of the neutrophil activator formyl-methionyl-leucyl-phenylalanine. Lung histology showed pulmonary edema and neutrophil accumulation.

Evidence that the antibody hypothesis may not be sufficient to explain all cases of TRALI comes from several sets of observations. All reported case series include cases in which neither HLA nor HNA antibodies could be demonstrated in any donor or in the recipient. WBC antibodies, particularly HLA Class I antibodies, are far more common in transfused blood components than is TRALI in recipients of these components. Lookback studies of implicated donors (e.g., donors who had a detectable antibody to an antigen specificity present in the TRALI patient) have found fewer cases of TRALI than would be expected from the known antigen frequencies in the recipient population.[14-16] In summary, not all cases of TRALI are associated with donor HLA or HNA antibodies and not all transfusions of components from donors with HLA or HNA antibodies cause TRALI.

The two-event hypothesis for TRALI (also termed the neutrophil priming hypothesis) states that TRALI is the result of two independent events.[12] The first event causes neutrophils to be primed, but not activated (first hit), and the second event causes activation of primed neutrophils (second hit). In the clinical setting, one of the events is caused by the patient's underlying clinical condition (infection, surgery, inflammation) whereas the other event is a consequence of transfusion.[12,22] Specifically, neutrophil priming or activation may occur with the infusion of substances in the plasma of the transfused product; these may be antibodies (via antigen–antibody reactions) or other biologically active substances (e.g., biologically active lipids) that accumulate in the blood product.

Support for the neutrophil priming hypothesis comes from the investigation of TRALI cases in which WBC antibodies could not be demonstrated. The largest series of such cases involved 90 TRALI events in 81 patients.[6] PLT concentrates or RBCs were judged to be the causative component in all but one of these cases. Of 28 cases investigated for antibodies, HLA or HNA antibodies were found in only 7 (7 positive donors in 104 tested donors). Elevated neutrophil priming activity was detected in implicated PLT concentrates when assayed by measurement of superoxide anion production of normal neutrophils. Elevated neutrophil priming activity was also found in the postreaction plasma samples of TRALI patients compared to prereaction plasma samples (n = 34), but no such difference was found in control patients (n = 10). Extraction of plasma from implicated PLT concentrates (n = 6) and TRALI patient plasma (n = 6) localized the neutrophil priming activity to lysophosphatidylcholines (lyso-PCs) and neutral lipids.

Experimental evidence in support of the neutrophil priming hypothesis comes from two models. In an ex vivo model, rats were treated with lipopolysaccharide (LPS) or saline control before the lungs were isolated, ventilated, and perfused.[22,23] After LPS treatment and perfusion with the supernatant of 42-day stored RBCs, there was increased pulmonary artery pressure and lung edema. Histology showed edema and intravascular neutrophil accumulation. These did not occur with saline treatment or with perfusion with supernatant from fresh RBCs. Lipid extracts of 42-day stored RBCs or purified lyso-PCs also produced injury in LPS-treated lungs.[23] Treatment of animals with a calcium channel blocker or a PLT-activating factor receptor blocker prevented lung injury. More recently, similar results have been obtained with 5-day stored PLTs but not with Day 0 PLTs.[22]

A second experimental model involved the culture of human pulmonary microvascular endothelial cells (HMVECs) and exposure to activated neutrophils.[24] HMVECs were activated with LPS and exposed to neutrophils before addition of neutrophil-activating lyso-PC. Killing of HMVECs was observed and was dependent on

LPS treatment and the dose of lyso-PC. Blocking antibodies to the endothelial cell adhesion molecule ICAM-1 or the neutrophil receptor CD18 inhibited HMVEC killing. HMVEC killing could also be reduced by the addition of neutralizing antibodies to the chemokines ENA-78, GRO-α, and interleukin (IL)-8. This model suggested that TRALI can result from a sequence of events involving endothelial activation (first hit), adhesion of neutrophils, and activation of adherent neutrophils (second hit).

The observed neutrophil dependence of experimental TRALI models has led to the hypothesis that neutrophil activation is a required intermediate event in the causation of ALI. Evidence that neutrophil priming and/or activation may not occur in all TRALI cases, however, comes from several sources. First, many cases of TRALI have occurred following the transfusion of FFP, in which elevated neutrophil priming activity has not been demonstrated.[17] Second, there are case reports of TRALI in severely neutropenic patients (absolute neutrophil count of <500). (L. Williamson, personal communication). Third, a report of unilateral TRALI occurring in a transplanted lung suggests that endothelial cell HLA antigens, not neutrophil antigens, can be the target of transfused antibodies.[25] Finally, it is unknown whether TRALI cases caused by HLA Class II antibodies occur through a neutrophil-mediated mechanism;[26] HLA Class II antigens are not expressed on resting neutrophils and there is controversy over whether such antigens are expressed on cytokine-activated neutrophils.

Other possible mechanisms for TRALI have been suggested, but these are not supported by clinical and experimental evidence. Monocyte antibodies and/or monocyte activation have been identified in some TRALI cases.[27] Cytokines or other yet to be identified biologic response modifiers that are present in blood components and which may increase with blood component storage may directly or indirectly activate or injure pulmonary endothelium. One possible candidate for this latter mechanism is IL-8, which has been shown to directly increase permeability of cultured endothelial cell monolayers.[28] A summary of our current understanding of TRALI pathogenesis is presented in Fig. 1.

The existing theories and evidence on the pathophysiology of TRALI leave a number of unanswered questions that require further research.

Question:

**What options are available for managing donors implicated in TRALI reactions?**

## APPROACHES TO DONOR MANAGEMENT

The Panel was presented with multiple approaches to donor management by the conference speakers. The Panel notes that donor management in TRALI is a complex issue with many decision points depending on the extent of the workup that is completed and the findings that are generated. Because of these factors, the Panel was not able to reach consensus on all aspects of this question. Instead, the Panel has attempted to delineate the issues logically and to indicate some of the critical decision points.

The Panel has defined a donor as *associated* with a TRALI reaction if one of his or her blood components was transfused during the 6 hours preceding the first clinical manifestation of TRALI. The Panel has defined an associated donor as *implicated* in TRALI if antibodies to an HLA Class I or II antigen HNA with specificity against an antigen present on the recipient's WBCs are detected.

The Panel discussed several approaches to donor management. The first approach was to take a uniform action for all donors associated with a case without performing any laboratory testing to identify an implicated donor. Possible actions would include deferring these donors from all future donation or restricting them to whole-blood donation with washed RBCs as their only allowable transfusable component. Another possible action would be to allow such associated donors to continue to donate but to place a flag in the donor record so that the donor would be deferred if subsequently associ-
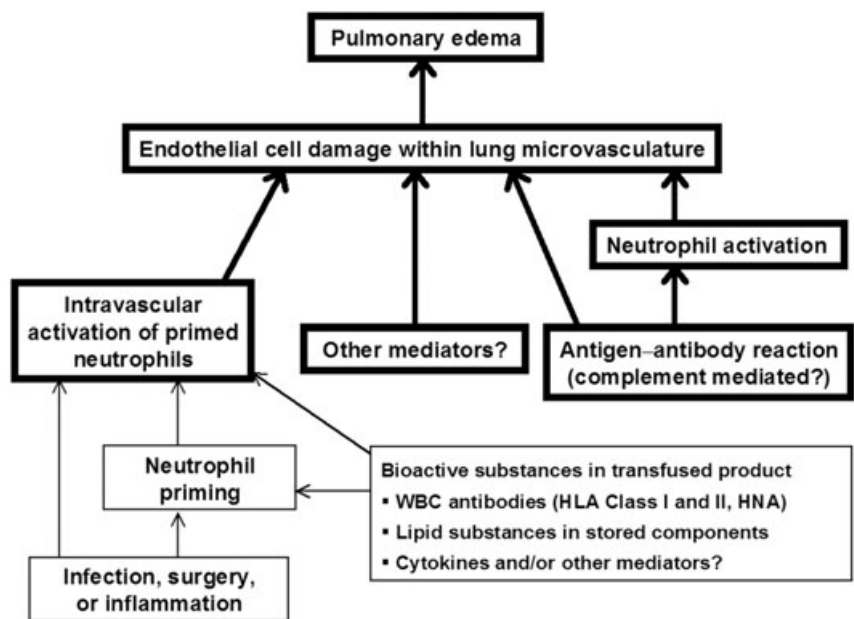


Fig. 1. Postulated pathophysiologic mechanisms of TRALI. The lightface arrows represent possible alternate pathways for neutrophil priming and activation in the two-event model.

ated with another TRALI case. It was noted that there were two problems with this donor record flagging approach. The first problem was that this might result in a subsequent case of TRALI that might have been preventable if more stringent donor management approach had been adopted. The second problem is based on a possible interpretation of Canadian informed consent requirements for recipients. In Canada, the informed consent process requires health-care providers to tell a patient about anything a reasonable person in the patient's position would want to know.[29] One possible interpretation of this requirement is that a recipient of a blood product from a "flagged" donor must be informed of the fact that the donor has been associated with a previous TRALI event. From a public trust perspective, this interpretation of consent law seems appropriate. Thus, if this interpretation is correct, then donor flagging would not be practical to implement.

With regard to TRALI cases, the Consensus Panel did not endorse either variation of the above approach. The Panel favored a more targeted approach to donor management based on the laboratory workup of *associated* donors to identify the implicated donor whose component was etiologic for the TRALI reaction. The purpose of identifying an *implicated* donor is to prevent the transfusion of future plasma containing donations from this donor, thereby possibly preventing TRALI in future recipients, and to avoid the unnecessary deferral of associated donors who are not implicated.

In the past, there have been at least two other reasons to perform laboratory workups in TRALI cases. First, some investigators have required positive serologic findings to designate a case as TRALI. Based on the Panel's proposed definition of TRALI, such positive laboratory findings are not necessary to make the diagnosis. Second, donor, product, and recipient laboratory testing have been important in elucidating the pathophysiology of TRALI. The Panel sees this as a highly desirable research goal, but not directly related to the question of donor management.

With regard to possible TRALI cases, the Consensus Panel believes that each blood collecting agency must decide if laboratory case investigation and donor management will be handled in the same fashion as for TRALI cases or if less stringent or otherwise different protocols should be adopted. The Panel notes that from a research rather than an operational perspective, it would be important to perform such investigations.

A flow chart detailing the steps involved in deciding to initiate and in performing a laboratory workup is presented in Fig. 2.
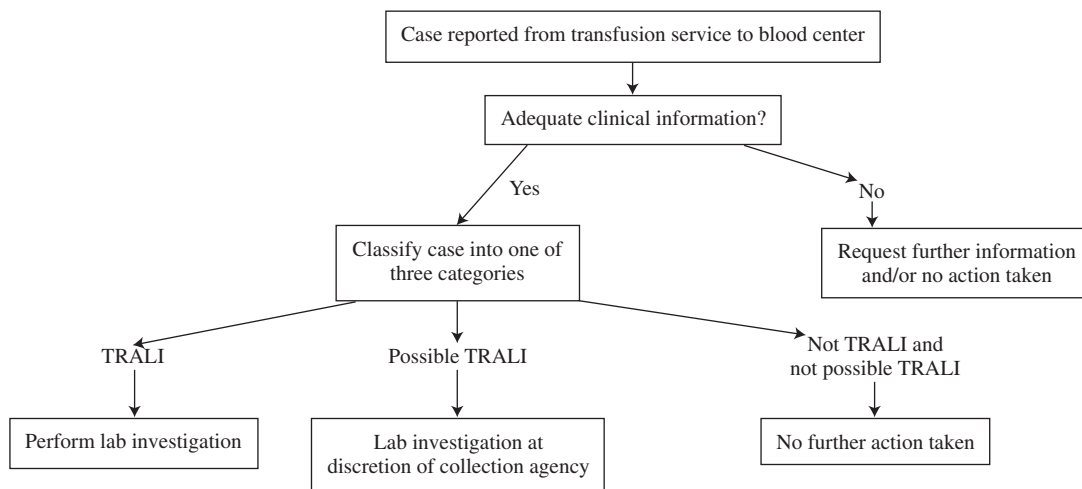


Laboratory investigations:

- Obtain recipient specimen from hospital:
  - Type for HLA Class I and Class II antigens (or procure information from patient record) and possibly for neutrophil antigens and/or use for performance of cross-match with donor specimens
- Obtain donor specimens either from blood component residual volume or from recall of donors (all donors or sequential testing algorithm)
  - Test donor specimens for HLA Class I, HLA Class II, and HNA antibodies
    - If HLA antibody identified, type for specificity
    - If HNA antibody identified, type for specificity if possible

**Fig. 2. Decision points for laboratory investigation of donors associated with a reported case of TRALI or possible TRALI.**

## LABORATORY WORKUP

### Prerequisites for performing a laboratory workup

A laboratory workup of a TRALI case is expensive and may require considerable effort by the blood center to contact associated blood donors to obtain necessary blood specimens. Furthermore, donors may experience anxiety and/or concern that their blood donation may have resulted in harm to a recipient. For these reasons, the Panel has concluded that before a blood center initiates a TRALI donor case investigation, the transfusing institution has the obligation of providing complete information on the TRALI case to the blood center. Information to be provided includes sufficient clinical data to confirm the TRALI diagnosis and to exclude other causes of ALI as well as the results of any laboratory tests performed to rule out other transfusion reactions (e.g., acute hemolytic transfusion reactions, sepsis). Data as to the storage age of the transfused components are also important for evaluating the neutrophil priming hypothesis of TRALI pathogenesis.

### Antibody and antigen testing

A complete case workup for the antibody etiology of TRALI requires both donor and recipient specimens. Donor samples should be tested for HLA Class I and II antibodies and for HNA-specific antibodies. There are now enough TRALI cases in which donors with HLA Class II antibodies have been implicated to make HLA Class II antibody testing a mandatory part of the workup.[27] If initial HLA Class I or II reactivity is identified, testing for the specificity of the HLA antibodies is required. If HNA antibodies are detected, ideally their specificity should also be determined. The Consensus Panel is aware, however, that the laboratory capability for determining HNA antibody specificity is more limited than for HLA antibodies.

There are two possible sources of donor specimens: residual volume from transfused components or fresh specimens obtained from recalling donors. It is likely that residual volume will not be available for all associated components. Furthermore, the Panel was not provided with any information to assess the adequacy of residual volume specimens for further antibody testing. (One speaker noted that archived samples have been associated with a high false-positive rate in neutrophil serology.) The Panel concludes that recall of donors will be necessary to complete many case evaluations.

A donor can be regarded as *implicated* in TRALI only if found to have antibodies to an HLA Class I or II antigen or HNA and either that antibody has specificity for an antigen present on the recipient's WBCs or there is a positive reaction demonstrated between donor serum and recipient WBCs (i.e., a positive cross-match). Therefore, a workup either requires the determination of the HLA type of the recipient, the neutrophil type of the recipient, or a cross-match between donor serum and patient cells. A recipient blood specimen containing adequate cells for cross-matching and/or for HLA and/or neutrophil typing should be obtained, as soon as possible after the TRALI episode by the transfusing facility and sent to the blood center. In some cases, depending on the patient's diagnosis, recipient HLA typing data may be available in hospital records.

Owing to the expense of donor testing and the logistics of recontacting donors, the question arises as to whether all donors associated with a TRALI case need to be investigated. The Panel notes that several speakers presented protocols for testing donors sequentially and discontinuing donor testing once a single implicated donor had been identified.[1] Examples of such protocols include beginning testing with donors whose components were administered closest to the onset of TRALI; donors of plasma, PLTs, cryoprecipitate, and RBCs, in that order; and multiparous female donors, other female donors, and then male donors. The Consensus Panel agreed that either a simultaneous investigation of all donors or a sequential approach to donor investigations would be reasonable but was not able to evaluate which of the sequential testing approaches would be preferable.

The specific approach to investigating donors could vary depending on the number of donors associated with a given case. The Panel, however, saw no reason to impose an arbitrary upper limit on the number of donors who should be tested in a TRALI case. Because laboratory investigation is confined to donors of components transfused in the 6 hours preceding TRALI onset, it should be rare that the number of donors in a given case will be unmanageable, even if it is necessary to investigate all associated donors. Patients receiving extraordinarily large numbers of components may have other temporally associated risk factors for ALI that preclude a specific diagnosis of TRALI and obviate the need to investigate the associated donors.

For detection of HLA antibodies, some techniques are more sensitive than others (e.g., Flow PRA is more sensitive than microlymphocytotoxicity).[1,26] For detection of HNA antibodies, the Panel noted that several speakers reported weak and/or nonspecific reactivity, the significance of which has not been defined.[1] Antibody detection methods should be standardized and sensitive, with screening panels selected to provide appropriate ethnic diversity and the ability to detect all clinically relevant antibodies.

Some cases of TRALI have been caused by antibodies in the recipient interacting with antigens on the donor cells. Most, if not all, of these cases occurred after transfusion of nonleukoreduced units.[10] Because of the low frequency of this cause of TRALI, the Panel considers this additional workup to be unnecessary for the purposes of donor management.

## Neutrophil priming activity

One set of investigators has identified increased neutrophil priming activity in stored blood components as an etiologic factor in some cases of TRALI.[6,12] This activity is assessed by adding plasma or serum (from a stored blood component or the recipient) to an assay system containing fresh neutrophils from volunteer donors and measuring the neutrophil respiratory burst after stimulation with formyl-methionyl-leucyl-phenylalanine.

There are two possible sample sources for measuring neutrophil priming activity in TRALI cases. One approach is to measure this activity in pre- and posttransfusion samples from TRALI patients and to thereby determine whether such activity has increased after a TRALI event. A second approach is to measure neutrophil priming activity in the residual volume of associated transfused components. Until neutrophil priming activity assays are more widely available and standardized (and accompanied by reference ranges for stored components), the Panel does not recommend such testing as part of a routine TRALI case investigation. The Panel encourages such workups in a research setting.

## DONOR MANAGEMENT

A donor is *implicated* in TRALI if found to have HLA Class I, HLA Class II, or HNA antibodies with a specificity directed against an antigen present on the recipient's WBCs or demonstrated by a positive cross-match. An implicated donor should either be permanently deferred from donation or have the plasma from future whole-blood donations diverted for fractionation, have no PLTs manufactured from the donation, have their RBCs washed (or frozen and deglycerolized) before transfusion, and be permanently deferred from future apheresis donation. This policy recommendation is based on documented instances in which donors with antibodies have been implicated in TRALI reactions in multiple recipients.[14,15] Although the evidence for this phenomenon is strongest for anti-HNA-3a (i.e., anti-granulocyte 5b), the Consensus Panel was of the opinion that a precautionary approach dictates taking this deferral action for any implicated donor, regardless of antibody specificity. This policy errs on the side of recipient safety in that it may lead to the deferral of some safe donors with common specificity antibodies that either may not have been the cause of TRALI in the recipient or, even if causal, might not cause TRALI in future recipients.

Multiple scenarios, as described in Table 5, may be encountered in performing a TRALI donor case investigation. Important variables that may influence donor management decisions include:

- Whether a recipient specimen has been obtained;
- Whether an implicated donor is identified; and
- In the absence of an implicated donor, whether specimens are obtained from all associated donors.

In the absence of a recipient sample (or of a known HLA type), it is not possible to identify an implicated

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**TABLE 5. Donor management decisions based on possible outcomes of TRALI donor case investigations**

| Case status regarding implicated donors | Classification of donors by antibody status | Recipient specimen antigen testing or cross-matching result* | | | |
|---|---|---|---|---|---|
| | | Result obtained | | Result not obtained | |
| | | All donors tested | Some donors not tested | All donors tested | Some donors not tested |
| Implicated donor found | Implicated donor | Deferral or washed RBCs only‡ | Deferral or washed RBCs only‡ | NA† | NA |
| | Other antibody-positive donors | TBD§¶ | TBD¶ | NA | NA |
| | Antibody-negative donors | Continue to donate | Continue to donate | NA | NA |
| | Nontested donors | NA | Continue to donate | NA | NA |
| No implicated donor found | Other antibody-positive donors | TBD¶ | TBD¶ | TBD¶ | TBD¶ |
| | Antibody-negative donors | Continue to donate | Continue to donate | Continue to donate | Continue to donate |
| | Nontested donors | NA | TBD | NA | TBD |

\* Recipient specimen may be typed (HLA and/or HNA) by a blood center or hospital or may be available from hospital records. Alternatively or in addition, cells from the recipient may be available for cross-matching with donor sera.

‡ NA = not applicable.

† The action is to permanently defer the donor from donation *or* to divert plasma from future whole-blood donations to fractionation, wash RBCs before transfusion, and defer from apheresis donation.

§ TBD = to be determined by those responsible for the blood supply in a given regional or national jurisdiction.

¶ The Consensus Panel believes that the decision with regard to these antibody-positive, nonimplicated donors needs to be determined by each jurisdiction. The Panel reached consensus that if a donor in this category had an HNA antibody of defined specificity, that donor should be managed in the same fashion as an implicated donor (see above). With regard to HLA antibodies, the majority of the Panel felt such donors should be allowed to continue to donate; the minority opinion was that they should be managed as an implicated donor.

donor by the Consensus Panel definition. In such cases, the decision to perform any donor antibody testing should logically be contingent on whether the institution's donor management policy would result in different outcomes for donors with or without detectable antibodies.

Donors with HLA or HNA antibodies not specific to a recipient antigen may be identified in cases in which another donor has been found to be an implicated donor or in cases in which no implicated donor is identified. The Panel was unable to reach full consensus as to proper management of such antibody-positive donors and believes that this decision should be made by those responsible for the blood supply in regional or national jurisdictions. The deliberations of the Consensus Panel, however, may be helpful to guide policy makers. First, the Panel reached consensus that deferral action should be taken for nonimplicated antibody-positive donors with anti-HNA antibodies with identified specificity. This recommendation is based on the positive lookback studies and mortality data associated with anti-HNA-3a, which the Panel generalized to other well-identified anti-HNA specificities.[14,17,18] This recommendation does not apply to anti-HNA antibodies that are nonspecific; the Consensus Panel emphasizes that the significance of such antibodies in TRALI is unknown and therefore should not be the basis of a deferral action in nonimplicated donors.

The policy with regard to nonimplicated donors with HLA antibodies was less clear because such antibodies may be found in approximately 10 to 20 percent of female donors and therefore could occur as a chance finding in a donor to a TRALI case.[1,13,26] For this reason, the Panel's majority opinion was that no action be taken on these donors (i.e., they be allowed to continue to donate), especially if the donor was ruled out as an implicated donor based on recipient antigen typing or donor–recipient cross-matching. The minority opinion within the Panel, however, was that the presence of an HLA antibody should lead to deferral action based on the premise that such a donor could potentially trigger TRALI in a future recipient with a corresponding antigen. A third potential option was to allow such donors to continue to donate but to flag their donor record. The Consensus Panel rejected this option based on the ethical and legal concerns previously discussed.

The Panel agreed that donors with negative tests for HLA and/or HNA antibodies may continue to donate whether or not the TRALI case investigation identified an implicated donor. To the Panel's knowledge, there is no evidence that such antibody-negative, TRALI-associated donors pose any additional risk to future transfusion recipients.

Question:

**Is there sufficient evidence, at this time, to recommend that any laboratory screening tests and/or other deferral measures be implemented to exclude donors to reduce the risk of TRALI?**

There are several sources of data indicating that TRALI is currently one of the leading causes of transfusion mortality in developed countries. As reported at this conference, TRALI appears to have been the leading cause of transfusion mortality from 2001 to 2003 in the US.[1,30] Based on reports to the FDA, there was an average of 16 fatal TRALI cases per year. In the UK, the SHOT program has attributed 24 deaths to TRALI over 7 years.[7] In Canada (excluding Quebec), 13 deaths were reported in nearly 3 years and two additional deaths over 4 years were reported by the Quebec hemovigilance system.[1,31] Furthermore, owing to underrecognition and underreporting of TRALI, these data almost certainly underestimate the extent of severe morbidity from TRALI.

Based on the available scientific evidence, the Consensus Panel first noted that there are no laboratory screening tests and/or other deferral measures that can be totally effective or specific for preventing TRALI. Because TRALI is a serious risk of transfusion, however, the Panel believes that a precautionary approach should be taken to policy development regarding decreasing the risk of TRALI. That is, in the absence of complete information, preventive measures should be taken even if the effectiveness of the measures is not fully known. There must be a valid rationale, however, for the specifically recommended measure(s) and there must be a balancing of the risks of not implementing the measures (e.g., the occurrence of morbidity associated with TRALI cases) against the risks of implementing them (e.g., the impact on blood availability and donor loss). Such a precautionary approach is extremely important in the context of blood safety, where delays or perceived reluctance to deal with past health risks have affected public trust and, in some jurisdictions, have threatened the integrity of the blood system.

The Panel noted that the two main hypotheses on the cause of TRALI lead to very different strategies for prevention. Although the antibody hypothesis dictates that excluding plasma from specific donors with pathogenic antibodies will prevent TRALI in recipients, the neutrophil priming hypothesis suggests that, independent of donor characteristics, providing reduced storage age or washed cellular blood products will prevent TRALI. Because there is evidence to support both mechanisms, it is likely that adopting a specific prevention strategy targeted at one of these mechanisms will be only partially effective. In addition, strategies directed against either mechanism are nonspecific and will reduce available blood components by excluding many safe donors (antibody hypothesis) or excluding safe products (neutrophil priming hypothesis).

The Panel agreed that there was currently one general strategy that should be adopted to reduce TRALI risk via

both mechanisms. That strategy is to encourage adherence to current guidelines for blood component utilization to minimize inappropriate use. This will reduce the risk of all transfusion-related adverse reactions. The Panel also recommended the deferral of donors positively implicated in a TRALI reaction (or restricting their transfusable components to washed RBCs only) because this would decrease TRALI risk by the antigen–antibody mechanism.

The Panel considered other proposals that could potentially decrease the risk of TRALI owing to the antigen–antibody mechanism. These proposals are based on restricting donations from certain groups of donors who are known to have higher rates of WBC antibodies, either owing to demographic characteristics (sex, parity) or through detection by previous testing.[1] Restrictions imposed on such donors could include: deferring donors from any type of blood donation; permitting whole-blood donation but diverting the use of plasma from FFP production to fractionation; deferring donors from the donation of components that have high plasma volume (e.g., apheresis plasma or apheresis PLTs); or a combination of the latter two approaches. Application of these strategies could be considered for any of the following groups of donors, listed in decreasing order of the number of donors affected:

- All female donors and all transfused male donors;
- All female donors;
- All previously pregnant female donors;
- All multiparous female donors
- Donors with previously demonstrated WBC antibodies (either by history or through screening programs initiated for this purpose).

Applying restrictions to any of these groups is nonspecific and would result in a loss of usable, safe blood components (i.e., components that will not cause TRALI) from large numbers of donors. The Panel noted that, in many jurisdictions, it may be unrealistic to defer any of the above groups of donors from whole-blood donation owing to the potential large impact of such a policy on blood availability. The Panel, however, was not provided with enough information to assess the impact on component availability or on donor behavior of deferring donors from apheresis donation or diverting plasma from FFP production. Accordingly, the Panel believes that more detailed assessments of the impact of these strategies versus their potential risk reduction need to be undertaken by those responsible for the blood supply in a given jurisdiction. These assessments should include:

- Estimates of the degree of risk reduction to be achieved by the strategy. This will be dependent on an analysis of national (or local) TRALI incidence data, TRALI morbidity and mortality data, association of TRALI with blood component type, and evaluation of whether cases were caused by the antigen–antibody mechanism.
- Impact of the strategy on the number and availability of donors and the supply of blood products.
- Impact on the donor population, including willingness to continue donating.
- Impact on the fractionated plasma pool owing to changed characteristics of input plasma (i.e., a larger percentage of plasma from female donors).
- The logistics of identifying donors in any of the designated groups. For example, identifying previously transfused males or multiparous females would likely require an additional donor screening question at each donation.
- The feasibility of mass screening for HLA (or HNA) antibodies.
- Financial costs and cost-effectiveness of implementation of any of the proposed strategies.

Such assessments will define whether the benefit of restricting donations or diverting plasma from any of the groups of donors mentioned outweighs the risk to the safety and availability of the blood supply. Different conclusions may be reached in different jurisdictions. For example, the Panel notes that a risk assessment carried out in the UK resulted in the decision to divert plasma from female donors away from FFP production.[7] The UK situation with regard to use of plasma, however, is relatively unique in that UK plasma is not sent for fractionation because of variant Creutzfeldt-Jakob disease safety concerns.

In addition to the strategies discussed above, the Panel was presented with three other prospective prevention strategies.

1. Use of "fresher" blood when transfusing patients at risk for TRALI. This strategy could be helpful in preventing cases attributed to the "two-event" mechanism if initial observations that neutrophil-activating substances accumulate in greater concentrations in blood components with increased storage time are confirmed. The Panel notes there is currently no consensus of which patient groups are at risk for TRALI.
2. Use of solvent/detergent plasma (if licensed and available) in place of FFP. This suggested strategy was based on the antigen–antibody mechanism and the fact that the pooling process would lower the titer of any pathogenic antibody and thereby potentially protect against TRALI. Surveillance data are not currently adequate to evaluate this hypothesis.
3. Use of PLT storage solutions if any become licensed. The value of this strategy is theoretically based on either the antigen–antibody mechanism (e.g., reducing the concentration of antibody by replacing plasma volume with additive solution) or the two-

event model (reducing the accumulation of neutrophil priming substances).

The Panel felt that there were insufficient data for the potential benefits of any of these additional proposed strategies to be determined at the present time but has listed these suggestions to promote future research.

Question:

**What further information and systematic research is necessary to better evaluate the issues of the epidemiology and pathophysiology of TRALI, to reduce the risk to transfusion recipients?**

The current understanding of the epidemiology, magnitude of risk, and pathophysiology of TRALI has been limited by several factors. These include the lack of a uniform definition of TRALI, the presence of other possible confounding causes of pulmonary edema in the same patients who develop TRALI, and the lack of well-designed prospective observational or randomized, controlled clinical trials of adverse transfusion events.

The international representation at this consensus conference indicates the worldwide motivation to reduce the risk of TRALI among transfusion recipients. Reducing risk must start with a concerted effort to better understand the epidemiology of TRALI. Knowledge of what constitutes a high-risk donor, blood product, and recipient should allow clinicians and researchers to better understand pathophysiologic mechanisms and to evaluate preventive actions and/or to appropriately target their efforts at risk reduction.

The Panel recommends a focused plan for epidemiologic research. First, a uniform, internationally accepted definition of TRALI is required to make possible reliable comparisons between different studies and expedite all research in this area. Second, the Panel recommends that resources for systematic epidemiologic research be targeted to multiple high-volume transfusion centers or regional networks of collaborating hospitals with varied recipient populations. Third, such centers need to develop an infrastructure that will allow systematic surveillance for all TRALI reactions. To the extent possible, there should be standardized specimen and data collection related to recipients, their associated donors, and transfused blood products. Rigorous postmortem pathologic assessments should be obtained for those patients who die from TRALI.

Research studies should be performed both on TRALI cases and on possible TRALI cases as an additional group using our proposed definitions. Initial research on TRALI cases should be targeted to determining the incidence of TRALI associated with the various types of blood components, the severity of outcomes, and the mortality rate. Research should also focus on the possible predictors of TRALI reactions among donors, blood products, and transfusion recipients. Studies should investigate the genetic, demographic, and clinical factors in the recipient that predispose to, or afford protection against, TRALI. In this context, additional lookback investigations of recipients of previous products from an implicated donor may prove useful. In the future, once risk factors are better defined, further epidemiologic research should include comparisons with control groups that may be matched by donor characteristics, blood products, or recipient characteristics to further define independent risk factors that contribute to TRALI occurrence. Research on possible TRALI cases should focus on some of the same issues as well as on developing criteria that can determine whether a possible TRALI case is due to transfusion or is the result of other ALI risk factors.

There are several additional clinical and epidemiologic questions that can be addressed. Because the neutrophil is thought to play a critical role in most models of pathogenesis, cases of TRALI in severely neutropenic patients should be subjected to extensive study. The possibility of mild cases of TRALI (e.g., cases not fitting the proposed definition) has been suggested and this should be further investigated. The mechanisms of fever and hypotension in TRALI have not been elucidated.

Meanwhile, the Panel also recommends that blood collection agencies develop their capabilities, share their expertise, and possibly, pool resources to allow for all cases of TRALI, particularly those occurring at centers involved in epidemiologic studies, to be fully investigated. These research investigations should, ideally, always include tests for antigen–antibody concordance, as well as neutrophil priming activity. In the context of such investigations, we recommend that standardized and optimized laboratory methods be developed with the aim of being able to more reliably compare findings from one laboratory to another. For example, the characteristics of "implicated" antibodies, including immunoglobulin class, subclass, and titer, should be defined and compared to those of antibodies that are transfused to patients who suffer no adverse event. With regard to the neutrophil priming model, investigators should seek direct evidence for neutrophil priming, neutrophil activation, and pulmonary endothelial injury, and address the issue of whether neutrophils are necessary for tissue damage to occur.

In the meantime, basic research on TRALI pathogenesis would be enhanced by the development of in vitro systems in which evidence of cellular activation and/or damage can be documented. This applies to both the antigen–antibody and the neutrophil priming models. Both clinical and laboratory studies should be undertaken to define antibody specificities (including immunoglobulin class, subclass, or interaction with complement) and antibody titers carrying the greatest risk of TRALI. If pathogenic WBC antibodies are identified, studies should

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**TABLE 6. Potential TRALI research questions**

Epidemiology and/or clinical
- What is the incidence of TRALI for each type of blood component transfused?
- What is the severity of outcome and the mortality of TRALI?
- What genetic, demographic, or clinical factors in the recipient predispose to or afford protection against TRALI?
- Does TRALI occur in neonates? If not, why not?
- Does a donor implicated in a TRALI reaction pose a risk to other transfusion recipients (i.e., did this donor cause TRALI in previous transfusion recipients)?
- How often are cases of possible TRALI due to transfusion vs. due to other factors for ALI?
- How often does TRALI occur in conjunction with circulatory overload?
- Are there mild forms of TRALI?

Pathophysiology—general
- Is the pathophysiology of TRALI and ALI or acute respiratory distress syndrome fundamentally the same?
- What is the frequency of TRALI in patients with neutropenia? What is the mechanism?
- What causes hypotension and fever in TRALI?
- Because both HLA antibodies and accumulated lyso-PC appear to be common in blood components, and many patients with possible predisposing factors are transfused, why is TRALI rare?
- Are cytokines or other biologic response modifiers involved in the pathogenesis of TRALI?

Pathophysiology—antigen–antibody mechanism
- What detection methods for HLA Class I, HLA Class II, and HNA antibodies should be used in the workup of TRALI cases?
- Are there antibody characteristics that increase or decrease the risk of TRALI (e.g., specificity, titer, class, subclass, ability to interact with complement)?
- What are the mechanism(s) by which antibodies cause pulmonary damage? Is this mediated through neutrophils?

Pathophysiology—neutrophil priming and activation mechanism
- What is the level of neutrophil priming activity in different types of blood components at various storage ages?
- What is the baseline level of such activity in various patient populations?
- What is the minimum concentration of lyso-PC necessary for in vitro priming and for clinically significant neutrophil priming?
- Do HLA and HNA antibodies result in neutrophil priming or activation?

Other
- Can animal models help in defining the pathophysiologic mechanisms of TRALI?

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explore their neutrophil priming activity and/or other mechanisms (e.g., complement fixation, cytokine release) that could lead to pulmonary damage.

Further investigations of the neutrophil priming hypothesis, including confirmation of findings by independent laboratories, should be encouraged. Studies are necessary to ascertain the neutrophil priming activity of large numbers of blood components in different anticoagulants at varied storage ages as well as baseline determinations of such activity in various patient populations. The correlation between lyso-PC concentration, in vitro neutrophil priming activity, and clinically recognizable TRALI should be investigated.

Further development of animal models will be essential to a better understanding of TRALI. Development of a standardized model that could be used to investigate multiple pathophysiologic mechanisms with the same experimental system should be encouraged. Based on the above discussion, the Panel has categorized some suggested research questions into various categories as presented in Table 6.

**ACKNOWLEDGMENTS**

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