*Vox Sanguinis* (2005) **89**, 1–10


REVIEW

# Transfusion-related acute lung injury (TRALI): a serious adverse event of blood transfusion

**J. Bux**

*DRK Blood Service West, Hagen, Germany*

Vox Sanguinis

**Background and Objectives** Analyses of fatal transfusion reactions in the UK and USA have shown that transfusion-related acute lung injury (TRALI) is among the most common causes of fatal transfusion reactions.

**Material and Methods** Review of the literature was used to analyse TRALI.

**Results** TRALI is characterized by acute respiratory distress and non-cardiogenic lung oedema developing during, or within 6 h of, transfusion. In atypical cases, TRALI can become symptomatic much later. TRALI must be carefully differentiated from transfusion-associated circulatory overload. In its fulminant presentation, TRALI can be clinically indistinguishable from acute respiratory distress syndrome occurring as a result of other causes. The severity of TRALI depends upon the susceptibility of the patient to develop a more clinically significant reaction as a result of an underlying disease process, and upon the nature of triggers in the transfused blood components, including granulocyte-binding alloantibodies (immune TRALI) or neutrophil-priming substances such as biologically active lipids (non-immune TRALI). Immune TRALI, which occurs mainly after the transfusion of fresh-frozen plasma and platelet concentrates, is a rare event (about one incidence per 5000 transfusions) but frequently (≈ 70%) requires mechanical ventilation (severe TRALI) and is not uncommonly fatal (6–9% of cases). Non-immune TRALI, which occurs mainly after the transfusion of stored platelet and erythrocyte concentrates, seems to be characterized by a more benign clinical course, with oxygen support sufficient as a form of therapy in most cases, and a lower mortality than immune TRALI.

**Conclusions** By virtue of its morbidity and mortality, TRALI has become one of the most serious current complications of transfusion. To prevent further antibody-mediated cases, the evaluation of TRALI should include leucocyte antibody testing of implicated donors. However, further studies are necessary for the prevention of this serious transfusion complication.

*Received: 18 January 2005,*
*revised 4 April 2005,*
*accepted 4 April 2005*

**Key words:** granulocyte, lung injury, neutrophil, pulmonary injury, TRALI, transfusion.

## Historical background and clinical significance

Until relatively recently, the respiratory system has not been considered to be an important target of transfusion injury, as transfusion-associated pulmonary injury has usually been attributed to circulatory overload. However, investigators have shown that non-cardiogenic lung oedema is also an important pulmonary complication of transfusion. Since Barnard's initial description in 1951 [1], non-cardiogenic lung oedema related to transfusion has been widely reported using various designations, including non-cardiogenic pulmonary oedema, pulmonary hypersensitivity and severe allergic pulmonary oedema. In 1983, Popovsky *et al.* [2] coined the term 'transfusion-related acute lung injury' (TRALI). With the first analysis of a large series of 36 patients

*Correspondence*: Jürgen Bux, MD, PhD, DRK Blood Service West, Feithstrasse 182, 58097 Hagen, Germany
E-mail: j.bux@bsdwest.de

reported in 1985 by Popovsky & Moore [3], TRALI was recognized as a distinct clinical entity.

The clinical significance of TRALI became evident in 1990, when Sazama published an evaluation of 355 transfusion-associated deaths reported to the United States Food and Drug Administration (FDA) in the period from 1976 to 1985 [4]. Of the fatalities, 15% were caused by acute pulmonary injury, representing the second most common cause of transfusion-associated death after ABO-incompatible transfusions. However, much of the emphasis in transfusion medicine in the 1990s remained on transfusion-transmitted viral infections. The British Serious Hazards of Transfusion (SHOT) initiative has consistently demonstrated, in its annual reports since 1996, that TRALI is one of the most common causes of transfusion-associated major morbidity and death. The results from other national haemovigilance programmes have also identified TRALI as a significant adverse transfusion event (Table 1). The reported mortality rates of TRALI vary, as follows: 6%, in the study of Popovsky & Moore [3]; 9%, in the SHOT report [5]; and 20%, in a recent re-evaluation of the French haemovigilance database [6].

## Clinical presentation

TRALI is best described as a clinical constellation of dyspnoea and bilateral pulmonary oedema (Fig. 1) that usually develops during, or within 6 h of, a transfusion. Hypoxaemia, fever, hypotension, tachycardia and cyanosis also often occur. Although the symptoms usually commence within 1–2 h of transfusion, some patients can develop dyspnoea as late as 48 h after transfusion (atypical TRALI) [10–12]. Radiological examination shows bilateral pulmonary infiltrates consistent with pulmonary oedema. Radiographs may be patchy in the first hours, with progression of the alveolar and interstitial infiltrates such that there can be a 'whiteout' of the entire lung. Radiological findings tend to be more remarkable than the physical ones. In its fulminant presentation, TRALI is indistinguishable from acute respiratory distress syndrome (ARDS), which is induced by causes other than transfusion. However, unlike ARDS (with its high mortality), the lesion in

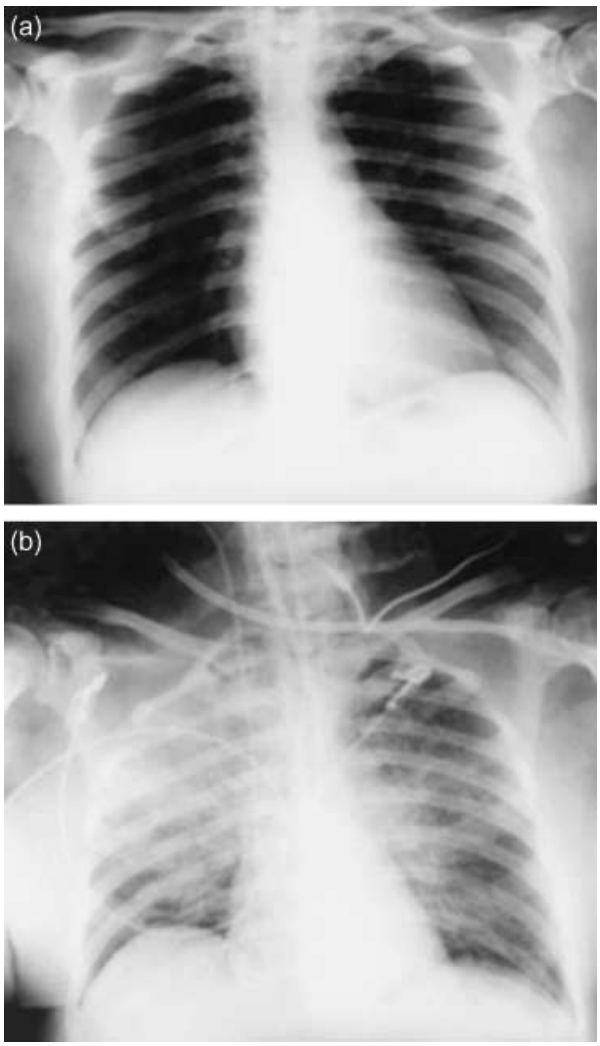


**Fig. 1** Chest radiograph, before and after blood transfusion, of a patient with transfusion-related acute lung injury (TRALI). Bilateral pulmonary infiltrates consistent with pulmonary oedema are an essential criterion for the clinical diagnosis of TRALI. Radiographs may be patchy in the first hours following transfusion, with progression of the alveolar and interstitial infiltrates such that there can be a 'whiteout' of the entire lung. Radiological findings tend to be more remarkable than physical findings. Reproduced from [31], with permission from Blackwell Publishing Ltd. Bux *et al.*

**Table 1** Transfusion-related acute lung injury (TRALI) in reports of haemovigilance networks

| | UK (SHOT) [5] | Germany (PEI) [7] | Denmark (DART) [8] | France (AFSSaPS) [6] | Canada (Quebec) [9] |
|---|---|---|---|---|---|
| Time-period | 1996–2003 | 1995–2002 | 1999–2002 | 1994–98 | 2000–03 |
| TRALI cases | 139 | 101 | 6 | 34 | 21 |
| Percentage of all adverse events | 7 | 3 | 7 | 0·15 | 0·5 |
| Mortality (%) | 9 (24[a]) | NR | NR | 20 | 9·5 |

[a]Including deaths possibly attributable to TRALI.
NR, not reported.

TRALI is, in most cases, transient and shows radiographical clearing of the infiltrates within 96 h in ≈ 81% of affected patients [3].

## Diagnostic criteria for TRALI and differential diagnosis

The first report of the French haemovigilance network, in 2003, did not include TRALI [13] as they, unlike SHOT, did not request it to be reported. Thus, in order to compare data between haemovigilance schemes, a definition of TRALI, based on agreed diagnostic criteria, has become imperative. A Working Party on Definitions of Adverse Transfusion Events has therefore been established by the European Haemovigilance Network (EHN). This group has suggested the following minimum requirements for the clinical diagnosis of TRALI: the occurrence of acute respiratory distress during or within 6 h of transfusion; no signs of circulatory overload; and X-ray evidence of bilateral pulmonary infiltrates (Table 2) [14]. These criteria are similar to those used by Popovsky & Moore [3]. The consensus panel of the North American 'Towards an Understanding of TRALI' Consensus Conference, held in Toronto in 2004, suggested a preliminary TRALI definition that included the same criteria as the EHN with the addition of clinical signs of hypoxaemia and the absence of additional risk factors for acute lung injury (ALI) (Table 2) [15]. In patients with ALI risk factors, TRALI should be considered 'possible'.

The differential diagnosis of patients who present with lung injury after transfusion includes transfusion-associated circulatory overload (TACO), cardiogenic oedema, allergic and anaphylactic transfusion reactions, and transfusion of bacterially contaminated blood components. Circulatory overload can result from hypertransfusion to individuals at risk, namely the very young or old recipient, in whom fluid infusion overwhelms the capacity of the left ventricle, resulting in pulmonary oedema. In contrast to patients with pulmonary oedema secondary to heart failure, patients with TRALI have normal central venous pressure and pulmonary wedge pressure. Allergic and anaphylactic reactions produce respiratory distress as a result of bronchospasm or laryngeal oedema, manifested by tachypnea, wheezing and cyanosis, and can occur after transfusion of very small volumes of blood components. Bacterial contamination manifests as fever, and hypotension and must be considered, especially in patients who have received platelets.

The EHN working party on definitions of adverse transfusion events has, for practical reasons, classified acute pulmonary injury in a transfused patient, with respiratory distress, as their leading symptom and without other conditions or factors that might be the cause of dyspnoea, into four categories: TRALI; TACO; allergic dyspnoea; and transfusion-associated dyspnoea (TAD). TAD should be diagnosed in a patient with acute respiratory distress, in temporal association with transfusion and in whom TRALI, TACO, allergic dyspnoea and transfusion-transmitted bacterial infections have been excluded [14].

**Table 2** Criteria for the clinical diagnosis of transfusion-related acute lung injury (TRALI)

TRALI definition of the European Haemovigilance Network (EHN) [14]

- Acute respiratory distress
- Bilateral lung infiltrations in the chest radiograph
- Occurrence during or within 6 h after completion of transfusion
- No evidence of transfusion-associated circulatory overload

Amendments by the TRALI Consensus Conference Committee in Toronto, 2004 [15]

- Hypoxaemia ($PaO_2/FiO_2$ < 300 or $O_2$ saturation < 90% or other clinical evidence)
- New acute lung injury (ALI) and no other ALI risk factors present including aspiration, multiple trauma, pneumonia, cardiopulmonary bypass, burn injury, toxic inhalation, lung contusion, acute pancreatitis, drug overdose, near drowning, shock and sepsis
- If one or more ALI risk factors are present, possible TRALI should be diagnosed

## Frequency

The exact incidence of TRALI is not known and different estimates have been made for its two forms. Antibody-mediated TRALI has been reported to be a relatively rare complication of transfusion, occurring at a rate of 1 in 5000 transfused units and in 1 of 625 patients transfused [3]. A recent study showed, for non-immune TRALI, an overall frequency of 1 in 1120 cellular blood components, with one incidence per 453 transfused platelet concentrates and one incidence per 4410 transfused red blood cells [16]. As many transfusion clinicians are not familiar with the syndrome, it is probable that TRALI is underdiagnosed and under-reported. This is equally true for mild cases of TRALI where mechanical ventilation is not required, and for severe cases of TRALI where transfusion may not be considered as possibly causative and which may be regarded as ARDS. In addition, TRALI can be misdiagnosed as TACO, as occurred in the French haemovigilance network [13].

## Forms of TRALI

There is growing evidence that TRALI can be triggered by two different factors: leucocyte antibodies; and non-immune neutrophil priming substances.

### Antibody-mediated (immune) TRALI

There is now little doubt that TRALI can be triggered by leucocyte antibodies. Unlike other immunologically triggered

transfusion reactions, the causative antibody in TRALI is usually identified in the donor and rarely in the recipient's blood. In two large series of TRALI, where pulmonary infiltrates were apparent in chest radiographs, leucocyte antibodies were detected in 61–89% of patients [3,17], and there are numerous case reports published by a variety of different authors describing antibody-mediated TRALI. In the recent report of the Center for Biologics Evaluation and Research (CBER) of the FDA, the majority of fatalities caused by TRALI were associated with leucocyte antibodies [18].

As early as 1957, Brittingham reported that leucocyte antibodies can induce post-transfusion pulmonary reactions [19]. A healthy volunteer was administered plasma, from two patients, that contained a weak leucoagglutinin: a mild respiratory reaction occurred after both infusions. Subsequently, 50 ml of whole blood from an alloimmunized patient with hypoplastic anaemia, and a strong leucoagglutinin, were given to the same volunteer who developed fever, hypotension, leukopenia and a marked respiratory reaction with associated marked bilateral pulmonary infiltrates on roentgenogram. A similar reaction was seen after the injection of 10 ml of sterile serum, from a patient with an even stronger leucoagglutinin, into a leukaemic patient. Induction of acute dyspnoea in a healthy individual after injection of a small volume of donor plasma containing HNA-3a antibodies is also known to the author of this review. Intravenous administration of an experimental gamma golublin concentrate, prepared from plasma containing leucocyte antibodies, to a healthy individual, resulted in severe pulmonary oedema [20]. McCullough *et al*. demonstrated, in 1986, by the use of $^{111}$indium-labelled granulocytes, that granulocyte antibodies can cause the pulmonary sequestration of granulocytes [21], and Seeger *et al*., in 1990, reproduced TRALI in an *ex vivo* rabbit lung model [22]. When the lung was perfused with plasma containing anti-HNA-3a (5b), accompanied by HNA-3a-positive granulocytes and rabbit plasma as a source of complement, severe pulmonary oedema occurred after a latent period of 3–6 h. If HNA-3a-negative granulocytes were infused, or if complement was not provided, the pulmonary reaction did not occur. Transient hypocomplementaemia and neutropenia have been reported in the course of TRALI reactions [19,23]. However, complement activation is not usually thought to occur as a result of granulocyte–antibody interaction, but may be caused by the activated neutrophils. Bux *et al*. [24] recently showed, in an *ex vivo* rat lung model, that HNA-2a antibodies can induce lung oedema without the addition of complement when HNA-2a-bearing neutrophils are added to the perfusate.

The leucocyte antibodies implicated in TRALI reactions are directed against human neutrophil alloantigens (HNA) and human leucocyte antigens (HLA) antigens, as well as against other less well-defined antigens expressed on neutrophils (Table 3). Description of the HNA antigens is beyond the scope of this review and has recently been published in detail [28,29]. Of the antibodies with known specificity, those directed against the HNA-1a (NA1), HNA-1b (NA2), HNA-2a (NB1), HNA-3a (5b) and HLA-A2 antigens have been reported most frequently [11,23,27,30–32]. More recently, antibodies to HLA class II antigens have been reported to be associated with TRALI [33]. However, HLA class II antigens are not expressed on resting neutrophils, and investigation of a case of suspected HLA class II antibody-mediated TRALI did not demonstrate the presence of HLA class II antigens on the surface of the patient's neutrophils [34]. Although binding of HLA class II antibodies to monocytes, with subsequent release of cytokines and activation of neutrophils, has been suggested as a possible mechanism [35], TRALI caused by the presence of HLA class II antibodies should be considered only as 'possible' until there are further supporting experimental data.

**Table 3** Leucocyte antigens implicated in transfusion-related acute lung injury (TRALI)

| | Old designation | Location | Ref. |
|---|---|---|---|
| Human leucocyte antigens (HLA) | | | |
| HLA class I | (HLA–A2 !) | | [25,26] |
| HLA class II | | | [33] |
| Human neutrophil alloantigens (HNA) | | | |
| HNA-1a | NA1 | Fcγ receptor IIIb | [30] |
| HNA-1b | NA2 | CD16b | [23] |
| HNA-2a | NB1 | NB1 GP/CD177 | [31] |
| HNA-3a | 5b | ? | [27] |

Most cases of TRALI are caused by donor antibodies, as the patient's antibodies can only react with the small number of neutrophils present in the transfused blood components. However, TRALI caused by antibodies in the recipient's blood occurred in 6% of cases in the study of Popovsky & Moore [3], and has also been reported by others [27,31]. In these cases, TRALI was mainly induced by the transfusion of whole blood or of non-leucocyte-depleted red blood cells. An inter-donor TRALI reaction (e.g. reaction of transfused antibodies with transfused leucocytes present in a blood component from a different donor) has also been described [11].

Blood components implicated in TRALI have included whole blood, red cells, fresh-frozen plasma, whole blood-derived platelet concentrates, apheresis platelet concentrates, cryoprecipitate [36], granulocytes [37,38] and intravenous immune globulin [39].

A frequent argument against antibody-mediated TRALI is that in 'look-back studies' some transfused patients did not develop TRALI, even though their leucocytes should have expressed the cognate antigens, as deduced from published antigen frequencies. There are several possible explanations for this, including:

(1) The recipient's zygosity for the antigen recognized by the antibody remained unknown although it can influence antibody-mediated neutrophil stimulation.

(2) Clinical condition of the transfused patient, which predisposed to a greater or a lesser extent the development of a symptomatic pulmonary transfusion reaction.

(3) Lack of clinicians' awareness of TRALI, poor documentation, so that mild TRALI reactions were not diagnosed and/or were not documented in the patient's records.

(4) The known discrepancy between those transfusions that are documented and those that are carried out, and the lack of traceability of blood components.

(5) The low frequency of many HLA antigens.

Furthermore, HLA antibodies usually represent a mixture of antibodies with different specificities and with different abilities to induce neutrophil aggregation. It is well known from proficiency testing that it is very difficult to identify all HLA antibody specificities in a single serum sample and not merely those that are prominent. As neutrophils express HLA antigens in lower numbers than lymphocytes [40], which are usually used for HLA antibody detection by cytotoxicity assays, the identified prominent HLA antibody specificities were not necessarily those responsible for TRALI triggering.

### Non-immune TRALI

In 11–39% of TRALI incidents, no leucocyte antibodies have been identified in either the patient or the donor [3,17]. This might be explained by incomplete antibody detection (see the section, below, on 'Diagnosis and laboratory investigation') or by the lack of awareness of TRALI. On the other hand, the working group of Silliman, Boshkov and Ambruso has published a number of study results indicating that, in predisposed patients, TRALI might be triggered by substances other than leucocyte antibodies [16,41]. They observed that stored platelet concentrates and red blood cells, at the time of outdate, contained a 'priming agent' that enhanced neutrophil NADPH oxidase activity in response to a soluble stimulus, formyl-Met-Leu-Phe [41]. After characterizing the neutrophil priming activity as lipids, especially lysophosphatidylocholines, Silliman *et al*. suggested that the infusion of biologically active lipids might be responsible for the occurrence of non-immune TRALI [41,42]. This priming activity was not present in fresh cellular blood components or in acellular plasma. Silliman *et al*. demonstrated a significantly higher level of neutrophil-priming activity in post-transfusion samples from patients who had TRALI reactions compared with their pretransfusion samples, and with pre- and post-transfusion samples from control patients with febrile or urticarial transfusion reactions [43].

The possible pathogenic significance of the neutrophil priming activity was demonstrated in a two-event animal model [44]. Rats were pretreated with endotoxin to simulate pre-existing sepsis. Their lungs were subsequently isolated and perfused, *ex vivo*, with saline, 5% fresh plasma, plasma from stored packed red blood cells (pRBC) from the day of collection (day 0) or from the day of outdate (day 42), and lipid extracts from day 42 plasma or purified lysophosphatidylcholines. Acute injury was not triggered in lungs perfused with saline, fresh plasma or plasma from day 0 pRBCs. Conversely, it was triggered in lungs perfused with day 42 plasma from pRBCs, lipid extract from day 42 plasma or lysophosphatidylocholines. Plasma from platelet concentrates, isolated on days 0 and 5 of storage and heat-treated before use, produced similar results [45].

In contrast to leucocyte antibodies, which can induce TRALI even in healthy individuals, the occurrence of non-immune TRALI requires a predisposing clinical condition in the patient, promoting the development of TRALI after the transfusion of cellular blood components containing neutrophil-priming factors ('two-hit model') [16]. In a recent large study of 90 non-immune TRALI reactions in 81 patients, those with haematological malignancies and cardiac disease were identified as patients at risk for non-immune TRALI [16]. With only one exception, all TRALI reactions were secondary to the transfusion of platelets and red cells. Mechanical ventilation was required in 3% of the patients and, in a single patient, TRALI precipitated death [16]. These numbers are lower than the ventilation and mortality rates of 72% and 6%, respectively, reported by Popovsky *et al*. for mainly antibody-mediated TRALI. In view of these findings, together with the large number of reports in the literature of mechanically ventilated patients with immune TRALI, and the frequent detection of leucocyte antibodies in fatal incidents of TRALI reported to the CBER of the FDA [18], it appears that leucocyte antibodies cause more severe TRALI reactions, whereas a mild reaction is characteristic of non-immune TRALI. The different incidences of 1 : 5000 and of 1 : 1120 per unit transfused (estimated for immune and non-immune TRALI, respectively) are in accordance with this opinion [3,16].

Characteristics of immune and non-immune TRALI are summarized in Table 4.

## Pathogenesis

In both immune and non-immune TRALI the neutrophil granulocyte is postulated as the effector cell. To understand the pathogenesis of TRALI, we must first consider lung transit of neutrophils under physiological conditions (Fig. 2). The pulmonary capillary network is geometrically complex, distensible and a vastly interconnected structure, containing a high concentration of neutrophils (≈ 28% of the blood granulocyte pool is located in the pulmonary circulation [46]), which are available on demand for host defence. In passing from arteriole to venule, a typical blood cell encounters 40–100 segments [47]. In contrast to red blood cells, which

**Table 4** Characteristics of immune and non-immune transfusion-related acute lung injury (TRALI)

| | Immune TRALI | Non-immune TRALI |
|---|---|---|
| Trigger | Leucocyte antibodies | Biologically active lipids |
| Main blood components implicated | Fresh-frozen plasma > platelet concentrates | Stored platelet concentrates > stored red blood cells |
| Occurrence | Can even occur in healthy individuals | Occurs predominantly in critically ill patients |
| Clinical course | Severe, often life-threatening, TRALI (70% mechanical ventilation) | Mild TRALI (oxygen support is usually sufficient) |

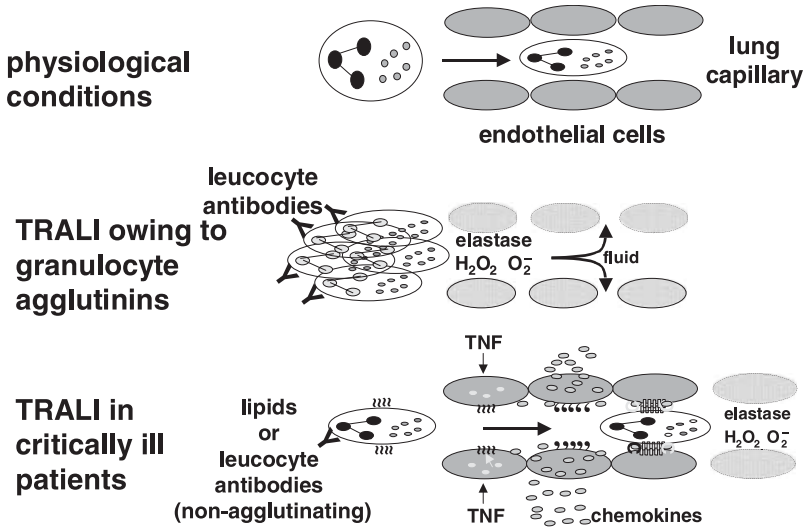


**Fig. 2** Neutrophil lung transit through lung capillaries under physiological conditions and in transfusion-related acute lung injury (TRALI). The average capillary diameter is similar to, or smaller than, the average size of a neutrophil. Therefore, after blood transfusion, antibody-mediated neutrophil agglutinates/aggregates become trapped in the first microvasculature, the lung capillary bed. Severe pulmonary transfusion reactions in patients and in healthy individuals are often caused by leucocyte antibodies that are able to agglutinate/aggregate neutrophils. In critically ill patients, in whom the endothelium of the lung capillaries and/or the patient neutrophils are preactivated by the underlying disease, neutrophil priming by biologically active lipids, or antibody–antigen interaction without aggregation, are sufficient to trigger transfusion-related acute lung injury (TRALI). The neutrophils stimulated by leucocyte antibodies or biologically active lipids release reactive oxygen radicals and toxic enzymes that damage the endothelial cells of the lung capillaries; this is followed by increased vascular permeability and exudation of fluid and protein into the alveoli, resulting in pulmonary oedema. TNF, tumour necrosis factor.

can easily change their shape and generally traverse the lung within a few seconds, the neutrophils travel in 'hops', with pauses, followed by rapid travel [47,48]. Because the average size of a neutrophil is similar to, or larger than, the average capillary diameter, it will often encounter a capillary segment that requires it to pause and change shape before passing through. This deformation time probably accounts for the longer transit times of neutrophils (mean 26 s, range 2 s–20 min) [48] so that neutrophil lung passage is mainly affected by their deformation time [48].

In view of the above, it is easy to imagine that granulocyte agglutinates (aggregates), induced by leucocyte antibodies in the transfused blood components (Fig. 3), become trapped in the first microvasculature they encounter following transfusion, namely the lung capillary bed. In fact, the most severe TRALI reactions were reported to be caused by leucocyte [i.e. granulocyte (neutrophil)] agglutinins. We now know that most of the leucocyte antibodies of the immunoglobulin G (IgG) class cause active neutrophil aggregation and do not agglutinate neutrophils passively.

Neutrophils stimulated by leucocyte antibodies or by biologically active lipids release reactive oxygen radicals and toxic enzymes that damage the endothelial cells of the lung capillaries. This is followed by increased vascular permeability and the exudation of fluid and protein into the alveoli, resulting in pulmonary oedema. Increased vascular permeability, attributed to leucoagglutinins in transfused, whole blood, has been demonstrated in a patient with TRALI. Shortly after the onset of the TRALI reaction, a contrast agent was injected into the main pulmonary artery. The late arterial and venous

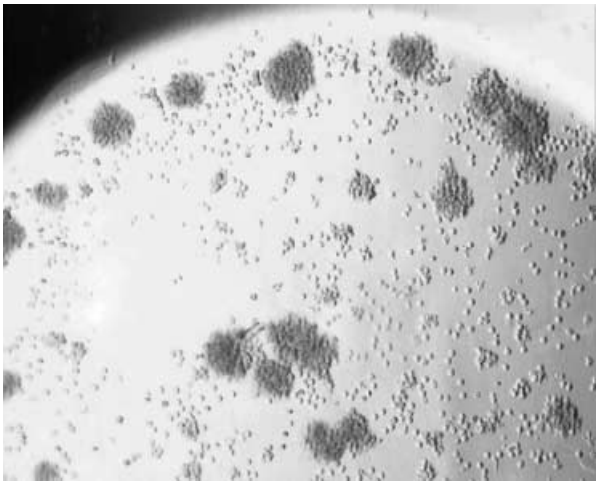

**Fig. 3** Granulocyte antibody-induced neutrophil agglutination/aggregation. Leucocyte antibody [anti-HNA-3a (5b)]-induced granulocyte agglutinates in a well of a microtest plate read on an inverted phase microscope (×100 magnification). The ability of leucocyte antibodies to cause granulocyte agglutination, i.e. to be a granulocyte agglutinin, can easily be determined in the granulocyte agglutination assay. The presence of granulocyte (leucocyte) agglutinins in donor blood has frequently been described in reports of severe transfusion-related acute lung injury (TRALI) reactions.

phases showed extravasation of the contrast agent into alveoli, in the presence of a normal wedge pressure, at the time of angiographic studies [49]. In another patient who died of immune TRALI within 2 h of the onset of symptoms, massive pulmonary oedema with prominent granulocyte aggregation within the pulmonary capillaries and amorphous proteinacous material within the adjacent alveolar spaces were reported at autopsy, which was performed 8 h after death [50]. Electron microscopy revealed capillary endothelial damage with activated granulocytes in contact with the alveolar basement membranes.

In critically ill patients, in whom the endothelium of the lung capillaries and/or the neutrophils are preactivated by the underlying disease, the binding of lipids, or an antibody–antigen interaction without aggregation, may be sufficient to trigger TRALI. Like non-immune TRALI, the triggering of mild TRALI is possibly also caused by neutrophil priming, but not by aggregating leucocyte antibodies.

There is an interesting report of TRALI in a patient with a single lung transplant, in whom, 10 weeks after transplantation, a transfused HLA-B44 antibody caused injury in the HLA-B44 expressing transplanted lung, but not in the patient's own, HLA-B44-negative, lung, as demonstrated by a total whiteout of the transplanted, but clear, native lung. This case report indicates that transfused HLA antibodies, which are not absorbed by recipient leucocytes and platelets, can also induce TRALI if they hit upon susceptible endothelial cells of the lung capillaries [51].

## Diagnosis and laboratory investigation

The diagnosis of TRALI is based primarily upon the clinical criteria listed in Table 2. It is important to determine that the pulmonary oedema is not caused by myocardial or valvular heart disease (i.e. cardiogenic), or by overhydration secondary to excess intake and/or inadequate output of fluid.

Laboratory investigation enables a diagnosis of immune TRALI by antibody detection and the prevention of further TRALI reactions by identification of the implicated donor and exclusion of their donated plasma-containing blood components. First, the recipient and the donors of fresh-frozen plasma and platelet concentrates transfused within the 6 h prior to the reaction should be identified, following which the female parous donors of all transfused components should be tested for leucocyte (i.e. HLA and granulocyte) antibodies. The antibody-screening tests should, as a minimum, include lymphocytotoxicity, granulocyte immunofluorescence and granulocyte agglutination using panels of typed granulocytes and lymphocytes. Tests should be performed in an experienced reference laboratory that participates regularly and successfully in national and international proficiency testing programmes. Appropriate conjugate should be used in immunofluorescence, in order to detect granulocyte-specific antibodies restricted to the immunoglobulin M (IgM) class. HNA-3a antibodies are often better detected by agglutination than by immunofluorescence [52]. For HLA antibody screening, antibody binding tests, such as enzyme immunoassays, flow cytometry or lymphocyte immunofluorescence, are preferred if possible, as they allow the detection of non-cytotoxic HLA antibodies that are otherwise missed. As HLA antibodies usually appear as mixtures of different specificities, making determination of all recognized antigens difficult, and because a number of granulocyte antigens are still unknown, a leucocyte crossmatch between donor serum and recipient leucocytes should be performed, whenever possible. A positive crossmatch confirms the diagnosis of immune TRALI. If the crossmatch is negative, non-immune TRALI may still be presumed in the appropriate clinical setting.

As TRALI reactions caused by leucocyte antibodies in the recipient have been described in incompatible granulocyte transfusions, the presence of leucocyte antibodies in the recipient should be excluded before transfusion, or compatibility should be checked in a leucocyte crossmatch.

## Treatment

Respiratory support should be as intensive as dictated by the clinical picture. Oxygen support is usually necessary (for example, in mild TRALI), and if hypoxaemia is severe, intubation and mechanical ventilation are important interventions (for example, in severe TRALI). If a recently transfused

patient complains of dyspnoea, careful surveillance is necessary if appropriate ventilatory support is to be given early. In a series of 36 patients with TRALI [3], all patients required oxygen support. Mechanical ventilation was required by 26 (72%) patients. Pressure agents may be useful in cases of sustained hypotension. The efficacy of corticosteroids is unproven, and the use of diuretics remains controversial. As the underlying pathology involves microvascular injury, rather than fluid overload, some patients benefit from fluid administration, whilst hasty and/or uncontrolled use of diuretics can be detrimental [3,10].

## Prevention

The donor of the implicated blood component in antibody-mediated TRALI is usually a multiparous woman [3]. This is primarily explained by antibody formation in female donors as the result of exposure to paternal leucocyte antigens from the fetus during pregnancy. HLA or lymphocytotoxic antibodies are present in the blood of 17–40% of parous women, granulocyte-reactive antibodies in 1–20%, and granulocyte-specific antibodies in 0·1–1% [53–55]. The percentage increases with increasing number of pregnancies. Payne demonstrated that 55% of women tested still possessed leucoagglutinins 3 years after initial testing and up to 8 years after the last potential exposure (pregnancy) [56]. The clinical significance of plasma from multiparous donors has recently been shown by Palfi *et al.* [57]. In a prospective, randomized controlled trial, 100 intensive care patients, judged to need at least 2 units of plasma, were randomly assigned to receive a unit of control plasma and, 4 h later, a plasma unit from a multiparous donor (e.g. three or more live births), or to receive the plasma units in reverse order. Transfusion of plasma from multiparous donors was associated with significantly lower oxygen saturation and higher tumour necrosis factor-α (TNF-α) concentrations than transfusion of control plasma. One patient presented typical signs of TRALI immediately after transfusion of the first plasma unit, which was from a multiparous donor who had formed granulocyte-specific antibodies.

The simple exclusion of multiparous donors would not be reasonable, because it would substantially reduce the donor pool [58]. The use of pooled solvent/detergent-treated plasma, or only male donor plasma, minimizes the TRALI risk of fresh-frozen plasma but does not solve the TRALI risk of cellular blood components. Screening of female donors, 6 months after the last pregnancy, for antibodies binding to neutrophils in immunofluorescence and agglutination tests using small panels of test cells covering the HNA-1a, HNA-2a, HNA-3a and HLA-A2 antigens, would result in the identification of most of the clinically relevant leucocyte antibodies. More sensitive techniques and panels, including low-frequency HLA antigens for HLA antibody screening, might result in the frequent detection of clinically irrelevant HLA antibodies. Fresh-frozen plasma and platelet concentrates from female donors with detectable granulocyte-reactive antibodies should not be transfused, whereas red blood cells resuspended in additive solution following the extensive removal of plasma may be considered acceptable. The latter approach will result in a reduced exclusion of blood donors whilst preventing severe antibody-mediated TRALI reactions.

The increasing use of leucocyte-depleted cellular blood products will reduce the incidence of TRALI caused by leucocyte antibodies in the recipient and possibly also non-immune TRALI reactions.

For non-immune TRALI, Silliman *et al.* have suggested careful selection of blood components for transfusion of those patients at risk, including the use of washed or fresh components [16]. However, such an approach raises many questions that must be answered before considering implementation. As the pathophysiology suggested by Silliman *et al.* for non-immune TRALI remains to be confirmed by other investigators, and because the clinical course of non-immune TRALI is usually benign, recommendations for preventive measures are difficult to make at present, especially in view of their potentially adverse consequences.

## Conclusions

By virtue of its morbidity and mortality, TRALI has become one of the most serious complications of transfusion today. Although our knowledge about clinical presentation and pathophysiology has significantly improved since its first description 50 years ago, many questions remain unanswered. Carefully documented cases reports are required, and close co-operation between blood services and haemovigilance networks on both national and international levels is necessary to answer the open questions and to establish preventive measures. TRALI has become too important to be ignored any longer.

## References

1 Barnard RD: Indiscriminate transfusion: a critique of case reports illustrating hypersensitivity reactions. *NY State J Med* 1951; **51**:2399–2402

2 Popovsky MA, Abel MP, Moore SB: Transfusion-related acute lung injury associated with passive transfer of antileukocyte antibodies. *Am Rev Respir Dis* 1983; **128**:185–189

3 Popovsky MA, Moore SB: Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. *Transfusion* 1985; **25**:573–577

4 Sazama K: Reports of 355 transfusion-associated deaths: 1976 through 1985. *Transfusion* 1990; **30**:583–590

5 Serious Hazards of Transfusion (SHOT): *Additional Cumulative Data 1996–2003*. www.shotuk.org

6 Renaudier PC, Vo Mai MP, Azanowsky JM, Breton P, Cheze S, Girard A, Hauser L, Legras JF, Rebibo D, Waller C, Ounnoughene N:

Epidemiology of transfusion-related acute lung injury in Gifit, the French hemovigilance database. A study of the French hemovigilance network. *Transfusion* 2004; **44**:23A

7 Graul A, Heiden M, Gräf K, Keller-Stanislawski B: Hämovigilanz in Deutschland – Berichte an das Paul-Ehrlich-Institut über Verdachtsfälle von Transfusionsreaktionen im Beobachtungszeitraum Januar 1995 bis Dezember 2002. *Transfus Med Hemother* 2003; **30**:232–238

8 Joergensen J, Taaning E: DART – a volunteer system of haemovigilance in Denmark. *Transfusion Alternatives Transfus Med* 2003; **5**:260–264

9 Robillard P, Nawej KI, Chapdeleine A, Poole J, Garneau N: Adverse transfusion reactions with respiratory signs and symptoms including TRALI in the Quebec Hemovigilance system: 2000–2003. *Transfusion* 2004; **44**:23A

10 Levy GJ, Shabot MM, Hart ME, Mya WW, Goldfinger D: Transfusion-related noncardiogenic pulmonary edema. Report of a case and a warning regarding treatment. *Transfusion* 1986; **26**:278–281

11 Lucas G, Rogers S, Evans R, Hambley H, Win N: Transfusion-related acute lung injury associated with interdonor incompatibility for the neutrophil-specific antigen HNA-1a. *Vox Sang* 2000; **79**:112–115

12 Zupanska B, Uhrynowska M, Konopka L: Transfusion-related acute lung injury due to granulocyte-agglutinating antibody in a patient with paroxysmale nocturnal hemoglobinuria. *Transfusion* 1999; **39**:944–947

13 Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, Janvier G, Herve P: Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. *Transfusion* 2002; **42**:1356–1364

14 European Haemovigilance Network (EHN): Definition of adverse transfusion events. Available from: http://www.ehn-org.net

15 Kleinmann S, Caulfield T, Chan P, Davenport R, McFarland J, McPhedran S, Meade M, Morrison D, Pinsent T, Robillard P, Slinger P: Towards an understanding of transfusion-related acute lung injury: statement of a consensus panel. *Transfusion* 2004; **44**:1774–1789

16 Silliman CC, Boshkov LK, Mehdizadehkashi Z, Elzi DJ, Dickey WO, Podlosky L, Clarke G, Ambruso DR: Transfusion-related acute lung injury: epidemiology and a prospective analysis of etiologic factors. *Blood* 2003; **101**:454–462

17 Popovsky MA, Haley NR: Further characterization of transfusion-related acute lung injury: demographics, clinical and laboratory features, and morbidity. *Immunohematology* 2000; **16**:157–159

18 Holness L, Knippen M, Simmons L, Lachenbruch P: Fatalities caused by TRALI. *Transfus Med Rev* 2004:184–188

19 Brittingham TE: Immunologic studies on leukocytes. *Vox Sang* 1957; **2**:242–248

20 Dooren MC, Ouwehand WH, Verhoeven AJ, von dem Borne AEGKr, Kuijpers RWAM: Adult respiratory distress syndrome after experimental intravenous gamma globulin concentrate and monocyte-reactive IgG antibodies. *Lancet* 1998; **352**:1601–1602

21 McCullough J, Clay M, Hurd D, Richards K, Ludvigsen C, Forstrom L: Effect of leukocyte antibodies and HLA matching on the intravascular recovery, survival, and tissue localization of 111-indium granulocytes. *Blood* 1986; **67**:522–528

22 Seeger W, Schneider U, Kreusler B, Witzleben E, Walmrath D, Grimminger F, Neppert J: Reproduction of transfusion-related acute lung injury in an ex vivo lung model. *Blood* 1990; **76**:1438–1444

23 Yomtovian R, Press C, Engman H, Kline W, Clay M, Hammerschmidt D, McCullough J: Severe pulmonary hypersensitivity associated with passive transfusion of a neutrophil-specific antibody. *Lancet* 1984; **22**:244–246

24 Bux J, Hardt O, Kohstall M, Sibelius U, Weissmann N: Reproduction of granulocyte antibody-mediated TRALI in an ex-vivo rat lung model. *Blood* 2003; **102**:94a

25 Wolf CFW, Canale VC: Fatal pulmonary hypersensitivity reaction to HL-A incompatible blood transfusion: report of a case and review of the literature. *Transfusion* 1976; **16**:135–140

26 Andrews AT, Zmijewski CM, Bowman HS, Reihart JK: Transfusion reaction with pulmonary infiltration associated with HLA-specific leukocyte antibodies. *Am J Clin Pathol* 1976; **66**:483–487

27 Nordhagen R, Conradi M, Drömtrop SM: Pulmonary reaction associated with transfusion of plasma containing anti-5b. *Vox Sang* 1986; **51**:102–107

28 ISBT Granulocyte Antigen Working Party: Nomenclature of granulocyte alloantigens. *Vox Sang* 1999; **77**:251

29 Bux J: Molecular nature of granulocyte antigens. *Transfus Clin Biol* 2001; **8**:242–247

30 Leach M, Vora AJ, Jones DA, Lucas G: Transfusion-related acute lung injury (TRALI) following autologous stem cell transplant for relapsed acute myeloid leukaemia: a case report and review of the literature. *Transfus Med* 1998; **8**:333–337

31 Bux J, Becker F, Seeger W, Kilpatrick D, Chapmann J, Waters A: Transfusion-related acute lung injury due to HLA-A2-specific antibodies in recipient and NB1-specific antibodies in donor blood. *Br J Haematol* 1996; **93**:707–713

32 Davoren A, Curtis BR, Shulman IA, Mohrbacher AF, Bux J, Kwiatkowska BJ, McFarland JG, Aster RH: TRALI due to granulocyte-agglutinating human neutrophil antigen 3a (5b) alloantibodies in donor plasma: a report of two fatalities. *Transfusion* 2003; **43**:641–645

33 Kopko PM, Popovsky MA, MacKenzie MR, Paglieroni TG, Muto KN, Holland PV: HLA class II antibodies in transfusion-related acute lung injury. *Transfusion* 2001; **41**:1244–1248

34 Kao GS, Wood IG, Dorfman DM, Milford EL, Benjamin RJ: Investigations into the role of anti-HLA class II antibodies in TRALI. *Transfusion* 2003; **43**:185–191

35 Kopko PM, Paglieroni TG, Popovsky MA, Muto KN, MacKenzie MR, Holland PV: TRALI: correlation of antigen-antibody and monocyte activation in donor-recipient pairs. *Transfusion* 2003; **43**:177–184

36 Reese EP, McCullough JJ, Craddock PR: An adverse pulmonary reaction to cryoprecipitate in a hemophilic. *Transfusion* 1975; **15**:583–588

37 O'Connor JC, Strauss RG, Goeken NE, Knox LB: A near-fatal reaction during granulocyte transfusion of a neonate. *Transfusion* 1988; **28**:173–176

38 Sachs UJ, Bux J: TRALI after the transfusion of cross-match positive granulocytes. *Transfusion* 2003; **43**:1683–1686

39 Rizk A, Gorson KC, Kenney L, Weinstein R: Transfusion-related acute lung injury after the infusion of IVIG. *Transfusion* 2001; **41**:264–268

40 Thorsby E: HLA antigens on human granulocytes studied with cytotoxic iso-antisera obtained by skin grafting. *Scand J Haematol* 1969; **6**:119–127
41 Silliman CC, Thurman GW, Ambruso DR: Stored blood components contain agents that prime the neutrophil NADPH oxidase through the platelet-activating-factor receptor. *Vox Sang* 1992; **63**:133–136
42 Silliman CC, Clay KL, Thurman GW, Johnson CA, Ambruso DR: Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. *J Clin Lab Med* 1994; **124**:684–694
43 Silliman CC, Paterson AJ, Dickey WO, Stroncek DF, Popovsky MA, Caldwell SA, Ambruso DR: The association of biologically active lipids with the development of transfusion-related acute lung injury: a retrospective study. *Transfusion* 1997; **37**:719–726
44 Silliman CC, Voelkel NF, Allard JD, Elzi DJ, Tuder RM, Johnson JL, Ambruso DR: Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. *J Clin Invest* 1998; **101**:1458–1467
45 Silliman CC, Bjornsen AJ, Wyman TH, Kelher M, Allard JD, Bieber S, Voelkel NF: Plasma and lipids from stored platelets cause acute lung injury in an animal model. *Transfusion* 2003; **43**:633–640
46 Peters AM: Just how big is the pulmonary granulocyte pool. *Clin Sci* 1997; **94**:7–19
47 Huang Y, Doerschuk CM, Kamm RD: Computational modeling of RBC and neutrophil transit through the pulmonary capillaries. *J Appl Physiol* 2001; **90**:545–564
48 Doerschuk CM: Neutrophil rheology and transit through capillaries and sinusoids. *Am J Respir Crit Care Med* 1999; **159**:1693–1695
49 Carilli AD, Ramanamurty MV, Chang YS, Shin D, Sethi V: Non-cardiogenic pulmonary edema following blood transfusion. *Chest* 1978; **74**:310–312
50 Dry SM, Bechard KM, Milford EL, Churchill WH, Benjamin RJ: The pathology of transfusion-related acute lung injury. *Am J Clin Pathol* 1999; **112**:216–221
51 Dykes A, Smallwood D, Kotsimbos T, Street A: Transfusion-related acute lung injury (TRALI) in a patient with single lung transplant. *Br J Haematol* 2000; **109**:671–678
52 Bux J, Chapman J: Report on the 2nd International Granulocyte Serology Workshop. *Transfusion* 1997; **37**:977–983
53 Bux J, Jung KD, Kauth T, Mueller-Eckhardt C: Serological and clinical aspects of granulocyte antibodies leading to alloimmune neonatal neutropenia. *Transfus Med* 1992; **2**:143–149
54 Clay M, Kline W, McCullough J: The frequency of granulocyte-specific antibodies in postpartum sera and a family study of the 6B antigen. *Transfusion* 1984; **24**:252–255
55 Skacel PO, Stacey TE, Tidmarsh CEF, Contreras M: Maternal alloimmunization to HLA, platelet and granulocyte-specific antigens during pregnancy: its influence on cord blood granulocyte and platelet counts. *Br J Haematol* 1989; **71**:119–123
56 Payne R: The development and persistence of leuko-agglutinins in parous women. *Blood* 1962; **19**:411–424
57 Palfi M, Berg S, Ernerudh J, Berlin G: A randomized controlled trial of transfusion-related acute lung injury: is plasma from multiparous donors dangerous? *Transfusion* 2001; **41**:317–322
58 Densmore TL, Goodnough LT, Ali S, Dynis M, Chaplin H: Prevalence of HLA sensitization in female apheresis donors. *Transfusion* 1999; **39**:103–106