

## Measures to prevent transfusion-related acute lung injury (TRALI)

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TRALI is one of the most serious transfusion reactions and is now the main cause of transfusion-related death, as was discussed in a previous international Forum [1]. Measures that may prevent TRALI have therefore been proposed. Since an important percentage of cases of TRALI are due to antibodies against HNA and/or HLA class I or class II antibodies in the donor blood, the following measures have been proposed: (1) the exclusive use of plasma from non-transfused male donors and (2) screening of donors with a history of blood transfusion and/or pregnancy for HNA and HLA antibodies before they are accepted as donors. It seemed of interest to investigate whether these measures are now taken and whether they are indications that they indeed lower the incidence of TRALI. To obtain such information, the following questions were sent to experts in the field:

### Question 1

Do you exclusively use non-transfused men as donors for FFP?

### Question 2

Do you reduce the amount of plasma in platelet concentrates by replacing it with platelet additive solution, and/or do you only use non-transfused male donors for apheresis platelets?

### Question 3

Do you screen donors who received transfusions and/or have been pregnant in the past for HLA class I and class II and HNA antibodies?

If the result is positive, do you exclude these donors for plasma-rich blood components?

### Question 4

Do you use any other measures that might prevent TRALI, for example pathogen-inactivated plasma or platelet concentrates.

If so, which are they and have you any data to indicate their efficacy?

### Question 5

If you do screen for HLA and HNA antibodies, which techniques do you use.

Do you foresee important technical developments in the near future?

### Question 6

Do you have data of the incidence of TRALI in your country, and if any of the above precautions have been taken, do you have data that this measure has reduced the number of TRALI cases?

Which percentage of TRALI cases you have seen was proven to be due to HLA and/or HNA antibodies?

Because the data provided by the participants vary considerably and because many interesting details are provided, it is essential that the answers are read to fully appreciate the value of this Forum.

In only two of the countries, no preventive measures are yet taken.

Different measures are taken with regard to plasma donors. In some countries/centres, only non-transfused male donors are used, but in others more complex measures have been adopted. In several countries, only male donors are used, but their transfusion history is not taken into account, since it has been found that the frequency of HLA antibodies was not higher in males with a, mostly remote, transfusion history [2, 3].

In France and Germany, all previously transfused donors are excluded, but women are accepted if they do not have HLA antibodies (France) or 'leucocyte antibodies' (Germany) if they have been pregnant. In countries where only male donors are used, an exception is made for donors of AB plasma for which a percentage of female donors have to be accepted. For further details, the reader is referred to the answers.

The use of additive solutions to decrease the amount of plasma in platelet concentrates is increasing. Although it is not yet generally used, in some countries part of the plasma of all platelet concentrates is replaced by additive solution. In general, additive solution is particularly used in pooled platelet concentrates. In some countries, the use of additive solution in 100% of platelet concentrates will soon be realized.

In addition, in many countries/centres, there are restrictions in the choice of platelet apheresis donors. In some, only male donors are accepted, or if female donors are accepted, they are screened for HLA antibodies if they have been pregnant. Again, the reader is advised to read the answers for further details.

In most countries, some categories of donors are screened for HLA antibodies and in some for HNA antibodies as well, for example female donors with a history of pregnancy. Screening for HLA antibodies is considered to be much more important than screening for HNA antibodies because the latter are rare (see Middelburg *et al.* page 244). It should of course be realized that the restrictions in the choice of donors described above make screening for HLA and HNA antibodies often superfluous. Several participants mention that testing for HLA and HNA antibodies in the donor(s) is part of the analysis of TRALI cases.

Although pathogen inactivation of plasma and/or platelet concentrates is applied in some of the countries/centres, it is not considered to be a measure to prevent TRALI, although several experts report that with solvent detergent-inactivated plasma (Octaplas), no cases of TRALI were observed. The only other preventive measure used is mentioned by Silliman *et al.* (page 258). In Rochester (USA) younger adults and some children with acute leukaemia, all neonates and infants undergoing cardiac surgery receive washed and prestorage leucocyte-reduced red cell and platelet concentrates in order to decrease the inflammatory effects of stored components and transfusion-related immune modulation. In the 15 years since this policy has been adopted, no cases of TRALI have been diagnosed in these patients. For the detection of HLA antibodies, the lymphocyte/cytotoxicity test (LCT) and the lymphocyte immunofluorescence test (LIFT) are still used, but in several countries have been replaced by a Luminex system, which

is an immunofluorescence assay with beads coated with HLA antigens.

For detecting HNA antibodies, the granulocyte agglutination test (GAT) and the granulocyte immunofluorescence test (GIFT) are still generally used, the results being verified by the monoclonal antibody immobilization of granulocyte antigens (MAIGA) assay in some centres. However, a combined Luminex test kit capable of detecting both HLA and HNA antibodies has been developed. At present, it does not include HNA-3 antigens, but since the gene has been sequenced, this problem is expected to be solved soon.

In all countries in which donors for fresh frozen plasma (FFP) have been restricted to males, hardly any TRALI cases have been diagnosed in recipients of FFP after this policy has been adopted, also in countries where the transfusion history of the male donors is not taken into account. No data are as yet available on the effect of preventive measures adopted with regard to platelet concentrates.

In many countries/centres, a series of TRALI cases have been serologically investigated. The percentage of cases in which HLA and/or HNA antibodies were detected in one or more of the donors varied from 28% to 78%, but the number of cases studied also varied considerably. As described before, it was found that in some cases, the HLA antigen against which the donor antibodies were directed was not part of the patient's HLA phenotype. All these results confirm, what was of course already well known, that a significant percentage of TRALI cases are not due to HLA and/or HNA antibodies in the donor blood.

In this regard, the observations mentioned by Silliman *et al.* (page 258) are of particular interest. A second important mechanism of TRALI, that is, a two-event mechanism, has been proposed in which the clinical situation of the patient is the first event and the transfusion of bioactive lipids and proteins that may accumulate in stored cellular components is the second event. The observation in Rochester, USA, that no cases of TRALI have been diagnosed in certain categories of patients who only received washed red cells or platelets from which bioactive material has been removed supports such a two-step process. This points to other preventive measures in seriously ill patients, that is, the use of cell concentrates that have not been stored for a long period of time, the removal of the supernatant from stored cell concentrates and the washing of red cells and platelets. In fact, in the Burn Unit of the University Hospital of Colorado (Denver, USA), red cells stored < 21 days or washed if stored longer have been used for a long time to decrease acute lung injury.

Thus, except in a few countries/centres, measures to prevent TRALI are taken, particularly with regard to plasma, as well as regarding platelets. By using male donors only, regardless of their transfusion history, has virtually

abolished TRALI due to FFP transfusions. Again, there is clear evidence that an important percentage of TRALI cases are not due to HLA and/or HNA antibodies in the donor blood. Possible further measures to prevent TRALI in such cases are discussed above. The LCT and LIFT for the detection of HLA antibodies have been replaced by a Luminex system in several countries/centres. It is a fluorescence assay using beads coated with HLA antigens. The GAT and IFT may soon also be abandoned when beads become available coated with HLA antigens and all the relevant HNA antigens as well.

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(1) In July 2007, the Australian Red Cross Blood Service introduced a policy of predominant FFP from male donors. Currently, 100% of groups A, B and O FFP, cryoprecipitate and cryo-depleted plasma issued to hospitals is manufactured from male donors. Only 80% of group AB FFP is sourced from male donors, with the remaining 20% from female donors. The transfusion status of male FFP donors is not recorded. The proportion of nulliparous to multiparous females

is unknown as we do not currently capture parity information.

(2) Currently, all apheresis platelets are suspended in donor plasma. This will be replaced by apheresis platelets in additive solution within the next 12 months. When this occurs, apheresis platelets in SSP<sup>+</sup> platelet additive solution will contain approximately 40% donor plasma.

Pooled platelets are prestorage leucocyte depleted and stored in additive solution (SSP<sup>+</sup>). However, they still contain 30% residual donor plasma.

Approximately 40% of the platelets supplied for transfusion are apheresis-derived, with the remainder being manufactured from pooled buffy coats. Approximately 70% of the plateletpheresis panel is male.

(3) The Australian Red Cross Blood Service does not screen donors with history of blood transfusions or pregnancy for HLA and HNA antibodies. However, donors who have been implicated in TRALI are tested for HLA class I and class II and HNA antibodies as part of the TRALI case investigation. If the implicated donor has any HNA-specific antibodies (e.g. HNA-3a or patient specific or high-titre HLA class I and class II antibodies), the donor is precluded from donating fresh components.

(4) Both pooled and apheresis platelets are leucocyte depleted. We are evaluating pathogen-inactivated technologies.

(5) We do not routinely screen donors for HLA and HNA antibodies. Only implicated donors in TRALI investigations are screened for HLA and HNA antibodies. For the detection of HLA class I and class II antibodies, we use Luminex technology single antigen bead kits. Screening for HNA antibodies is performed using flow-based granulocyte immunofluorescence and granulocyte agglutination antibody tests.

Cases of TRALI are reported to the Australian Red Cross Blood Service for laboratory investigation and donor follow-up. Following the introduction of predominant male FFP in July 2007, there has been a reduction in the number of TRALI cases reported to the Blood Service. Of note, there have been no cases reported of TRALI where FFP is the only implicated component since July–December 2009, although there have been some cases with multiple component types including FFP and cryoprecipitate.

(6) A few cases with HLA and HNA antibodies directed against patient antigens have been found. However, the more common scenario is the detection of HLA class I or class II antibodies in the donor that are not directed against patient antigens, but still result in donor deferral as they have been detected in the context of a suspected case of TRALI.

TRALI cases reported to the Australian Red Cross Blood Service	2006	2007	2008	2009	2010	Jan-Jun 2011
Total suspected TRALI cases	42	27	30	27	17	9
Number of cases that fulfilled TRALI case definition	14	7	11	7	6	2
Number of cases that fulfilled possible TRALI case definition	21	20	16	16	8	5
Other diagnosis	7	0	3	4	3	2
Number of cases (% of total) with HNA antibodies directed against patient antigens	Data not available	0	1 (3%) Anti-HNA-3a	1 (3%) Anti-HNA-1a	0	0*
Number of cases (% of total) with HLA antibodies directed against patient antigens	Data not available	0	0	1 (3%) HLA class I	2 (11%) HLA class II	0*

\*Still under investigation.

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## Background

In the whole of Austria, different types of FFP are used: mostly, SD-treated plasma (70.9%), in a minor percentage quarantined (21.9%) and methylenblue-treated plasma (7.2%) [1]. The incidence rate of TRALI documented in the nationwide haemovigilance system is low. Like in many other countries, underreporting cannot be excluded. My institute, the Central Institute for Blood Transfusion and Immunology (ZIB), part of the University Hospital Innsbruck, serves the Austrian federal state of Tyrol (700 000 inhabitants) including a University Clinic with more than 1500 beds providing all major surgical interventions and conservative therapies. In our centre,

mostly quarantined and methylenblue-treated FFP are used.

Ad 1) In 2005, we observed in the University Hospital Innsbruck a life-threatening TRALI in a patient undergoing coronary bypass operation. He had been treated at the end of the surgery with a quarantined FFP of a female donor who then turned out to have HLA antibodies. HNA antibodies were not tested for. Because of this severe case, we changed to 100% plasma from men without a transfusion history. In the last years, we did not observe any TRALI cases but got more and more into trouble to gain sufficient amounts of quarantined male-only plasma. That is also one of the major reasons why we introduced methylenblue-treated plasma.

Ad 2) In our centre, we prepare exclusively platelets in additive solution. Female platelet apheresis donors are asked for pregnancies, and if they confirm this, they are tested for HLA antibodies. When negative and if there is no history of involvement in any TRALI case they can serve as donors.

Ad 3) We do not perform this form of screenings.

Ad 4) As stated before, SD plasma is widely used in Austria. So far, the preparation of pathogen-inactivated platelets was validated in three Austrian Blood Centers. But at the moment, these components are not routinely used, so there are not yet data available for these products with regard to TRALI.

Ad 5) In our institute, we only screen for HLA class I/II antibodies, using an ELISA method. As there are no commercially HNA-antibody-detection systems available which are able to detect HNA 3a, we do not screen for HNA antibodies but hope that we can change this in the nearer future.

Ad 6) For whole Austria 2009 [2] (haemovigilance data of 2010 not yet available), two TRALI cases were reported. In the same year, 67 100 units FFP and 38 500 therapeutic doses of platelets were transfused [3]. The first TRALI case following the transfusion of two packed RBC had a lethal outcome, no FFP was transfused, and no immunological confirmation was gained. The second case was correlated with plasma transfusion, the patient only presenting with slight symptoms. The case was confirmed as TRALI. In Innsbruck, we observed from 2006 to October 2011, corresponding to 71 831 plasma and 29 056 platelet transfusions, only one suspected TRALI case that could not be confirmed.

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### Question 1

We use males, and for AB plasma, males and females without a history of pregnancy, for plasma destined for transfusion. All donors are asked about transfusion in the past 12 months and deferred for 1 year post-transfusion. However, we do not have an additional question regarding transfusion as a TRALI prevention measure. In a study of HLA class I and class II antibody frequency performed on donors deferred for a variety of reasons, such as geographic vCJD risk, we did not find an increased frequency of HLA antibodies in individuals with a history of transfusion. Many of these individuals had a remote history of a single transfusion episode. There was a marked increase in antibody frequency in donors with a history of pregnancy [1].

### Question 2

For apheresis platelet donations, we use male donors and female donors who do not have a history of pregnancy. We will exceptionally use female donors with a history of pregnancy if they are the only HLA or HPA donors available for a given patient and the treating physician is aware and would like to prioritize matching over TRALI risk reduction. For buffy coat platelets, we suspend the platelets in the plasma from a male donor.

### Question 3

We do not routinely screen donors for HLA and HNA antibodies. When a possible case of TRALI occurs, we perform antibody testing on donors who provided components received by that patient. These donors may be deferred from future donation, depending on results.

### Question 4

We have not implemented any pathogen-reduced components at the moment. However, SD plasma will soon be available for selected patients in Canada. Additionally, a clinical trial of pathogen-inactivated platelet concentrates is being planned. After switching from the platelet-rich plasma (PRP) method of production to the buffy coat method of production, there is a reduction in the volume of plasma contained in the red cell units. It is possible that this will have some impact on the frequency of TRALI.

### Question 5

We are not routinely screening for HLA or HNA antibodies.

### Question 6

We have some data on the incidence of TRALI, which appears to be decreasing since prevention measures were implemented [2]. In 2007, there were 36 reported cases that met the Canadian Consensus Conference definitions of definite and possible TRALI. This number fell to 19, 12 and 10 cases in 2008, 2009 and 2010, respectively. The decline was most marked for plasma-associated cases, but occurred for all components.

Focusing on the 3-year period from 2008 to 2010, there were 41 reported cases of definite and possibly TRALI. Using a flow cytometric microbead assay, donor HLA class I and/or class II antibody was detected in 14 of 26 completed cases; in six of the 14 (43%), the antibody was directed against a recipient cognate antigen. Donor HLA antibodies were also detected in seven of 10 cases with incomplete testing; in four of the seven (57%), the antibody was directed against a recipient cognate antigen. We have not yet performed HNA antibody detection on these cases, as we work to validate methods.

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### Question 1

No.

### Question 2

We have not reduced the amount of plasma in platelet concentrates by replacing it with platelet additive solution.



We use male and female donors for apheresis platelets.

### Question 3

The donors who received transfusions and/or have been pregnant in the past are not screened for HLA class I and class II and HNA antibodies.

### Question 4

We have some ways to prevent TRALI including using leucocyte-reduced red-blood-cells, washing blood cells and training for reasonable blood usage including reducing FFP and so on. But we have no data about the efficacy by now.

### Question 5

We have several techniques to screen for HLA and HNA antibodies, but they are not universal in our country now.

For HLA:

- (1) Complement-dependent cytotoxicity
- (2) Enzyme-linked immunosorbent assay (ELISA)
- (3) Lymphocyte/neutrophil immunofluorescence test flow cytometry (Lift-FCM)
- (4) Flow beads

For HNA:

- (1) Complement-dependent cytotoxicity
- (2) Lymphocyte/neutrophil immunofluorescence test flow cytometry (Lift-FCM)

A good technical may need be automated, large scale and sensitive, but all the ways above cannot match them. So we hope there will be a new method that can be found in the near future.

### Question 6

We have not the data of the incidence of TRALI in our country now. But we found some cases about TRALI from the clinical reports. According to statistics, there are 33 cases who diagnosed as TRALI, among which 15 patients were died at last. Six of them are identified to be transfused by women's plasma who have been pregnant in the past. There is also one patient who was transfused from male and non-transfused donors whose HLA antibodies were negative. Twelve of them are definitely diagnosed as TRALI according to American diagnostic criteria of TRALI; 14 cases are probably TRALI and seven cases are misdiagnosis.

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### Question 1

The blood transfusion service is decentralized in the Czech Republic. There are 53 independent, mostly hospital-based blood establishments (BE). There is no general recommendation for TRALI prevention. The Board of the Czech Medical Society for Transfusion Medicine performed the survey whether BEs enforced any selection criteria in donors of plasma for clinical use in 2010: 48.2% of plasma for clinical use is produced from non-selected donors. Donor selection is done (51.8% of plasma) in different ways:

- 9.4% of plasma ... non-transfused men only
- 11.0% of plasma ... non-transfused men + non-transfused and never-pregnant women
- 30.3% of plasma ... men only (transfusion non-questioned)
- 1.1% of plasma ... other selection

### Question 2

Platelet concentrates are produced by 18 BEs in the Czech Republic (11 of them produce also platelets by apheresis). Plasma in platelet concentrates is reduced in several centres – in general, circa 1/6 of therapeutical doses are 'plasma-depleted', but accurate data are not available.

### Question 3

Only two centres producing platelets from apheresis do any screening: one centre screens women after pregnancy and the other screens both donors after transfusion and women after pregnancy.

### Question 4

No other measures to prevent TRALI are applied.

### Question 5

LCT and/or ELISA tests are used if the testing is done.

### Question 6

Nine cases of TRALI were reported in 2010 (RBC: 4, plasma: 3, platelets: 2); results of HLA and/or HNA antibodies testing in donors are not included into report unfortunately. As far as above mentioned measures were introduced successively, their impact cannot be proven. High percentage of TRALI cases linked to transfusion of RBC (plasma removed) supports our feeling, that implemented measures were at least partially effective. The survey of TRALI reactions in 2010 is performed at this time.

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### Question 1

The Finnish Red Cross Blood Service introduced Octaplas® (Octapharma, Vienna, Austria) as the exclusive FFP in Finland in 2007. Octaplas® is a pooled pathogen-reduced pharmaceutically manufactured fresh frozen plasma for transfusion. Due to pooling allergenic substances, HLA and HNA antibodies are diluted out. Therefore, significantly less adverse reactions are observed in patients transfused with Octaplas® than in patients transfused with regular FFP.

Since 2007, regular FFP or cryosupernatant plasma has not been used for transfusion in Finland. Octaplas® for the Finnish market is produced both from recovered and from apheresis plasma prepared in Finland. Plasma is collected from both female and male donors.

### Question 2

Platelets are prepared by the buffy coat method and suspended in additive solution. HLA- and HPA-typed platelets are collected by apheresis and suspended in donor plasma. Both female and male donors are accepted as plasmapheresis donors. HLA- and HPA-typed platelets account for < 2% of total platelet components issued.

### Question 3

In Finland, donors who have received transfusions or been pregnant are not routinely screened for HLA and HNA antibodies.

### Question 4

As described above, only Octaplas® is used for transfusion.

### Question 5

Blood and plasma donors are not routinely screened for HLA or HNA antibodies.

### Question 6

Haemovigilance data show that no TRALI cases related to fresh frozen plasma transfusions have been reported since

the introduction of Octaplas® as the exclusive FFP for transfusion, whereas there has been no change in the rate of TRALI reactions related to red cell transfusions. Number of reports to the national haemovigilance system has stayed stable as based on the rate of reported mild and serious adverse reactions related to blood transfusions.

## Cases and rate of TRALI reactions in Finland in 2004–2010

Blood component transfused	Number of TRALI cases reported	Cases of TRALIs per 100 000 components transfused
2004–2007 (first half)		
Red cells	5	0.5
Platelets	0	
FFP	5	2.9
FFP and red cells or platelets	2	
2007 (second half)–2010		
Red cells	4	0.4
Platelets	1	0.6
Octaplas®	0	
Octaplas® and red cells or platelets	0	

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### Question 1

In France, regulations exclude all previously transfused individuals from blood donation. Thus the EFS (Établissement Français du Sang) is exclusively distributing, as single-donor fresh frozen plasmas (FFP), units obtained from non-previously transfused donors and since 1 January 2010 from non-nulliparous female donors testing negative for HLA class I and class II antibodies. Units of solvent detergent-treated plasma (SDP) are obtained from pools of a hundred individual plasmas. Each pool must test negative for HLA class I and class II antibodies.

### Question 2

Approximately 70% of platelet concentrates delivered in France (both aphaeresis and pooled whole-blood-derived) are presently plasma-reduced using additive solutions.

Automated methods for the preparation of mixed, whole-blood-derived, platelet concentrates (MPC) have been generalized in June 2011 with only marginal exceptions. These procedures entail plasma reduction using additive solutions. Thus, all CP used have residual amounts of plasma varying from 20% to 35% of the final suspension volume according to the additive solution used, respectively, SSP+® (MacoPharma, Mouvaux, France) or Intersol® (Travenol, La Châtre, France) [1].

Mixed whole-blood-derived platelet concentrates (MPC) are made from 5 to 6 individual whole-blood donations; they must not include donors known to have HLA antibodies.

Never-transfused men or never-transfused never-pregnant women are preferred for the donation of apheresis platelet concentrates (APC). Occasionally, if non-nulliparous women are requested to give platelets, they must be negative for HLA antibodies. This policy was recommended since the early beginning of 2010 and made compulsory on 1 January 2011.

#### Question 3

As mentioned above, all previously transfused individuals are excluded from blood donation and non-nulliparous women are not used for plasma-rich blood components obtained from a single donor unless they tested negative for HLA class I and class II antibodies.

#### Question 4

All individual FFP delivered in France are provided by apheresis and are pathogen-attenuated. Platelet concentrates are pathogen-attenuated (Intercept® Blood System; Cerus Europe BV, Amersfoort, the Netherlands) in Alsace as well as at the Reunion, Guadeloupe, Martinique and Guyane [2]. No data are currently available to evaluate the efficacy of these inactivation methods in TRALI prevention.

#### Question 5

Screening and identification of HLA class I and class II antibodies are done in regional EFS histocompatibility laboratories. A national strategy has been set up which leads to recommend the Luminex technology for this purpose. This decision relies upon the higher sensitivity of this technology as compared to the ELISA method. Two types of reagents, both based on the use of multiantigen bead kits, were selected for this purpose (i.e. One Lambda and Geneprobe Kits).

The EFS HLA Laboratory network has conducted a large-scale study to define the more appropriate cut-off values for each reagent in order to allow interlaboratory comparisons and standardization. Since only a screening test is currently performed on donor samples, the cut-off was set to discriminate at best the potentially dangerous strong

antibodies from the weak positive results, considered irrelevant for TRALI prevention.

In laboratory investigation of clinical TRALI, the specificities of positive samples were identified using a Luminex method using microbeads coated with a HLA single antigen. Screening for HNA antibodies are performed in two laboratories using the granulocyte agglutination test (GAT) [3] and the granulocyte immunofluorescence test (GIFT) [4]. HNA-1, 2, 4 and 5 specificities are studied by the monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA) technique [5]. HNA-3a antibodies are serologically detected on granulocytes of known HNA-3 phenotype. Automated methods allowing simultaneous detection of HLA and HNA antibodies are awaited.

#### Question 6

TRALI cases reported to the French Medicines and Healthcare Products Regulatory Agency (AFSSAPS) for 2007 and 2008 were validated by a working Group and have been recently published [6]. In this series, 62 TRALI cases and 23 possible TRALI were identified. When the implicated blood product could be accurately determined, single-donor high-plasma-volume components were involved in half the cases and carried the highest risk per unit, 1:31 000 for single-donor plasma FFP units and apheresis platelets as well. In this study, no TRALI case could be definitely attributed to the transfusion of SDP (>200 000 units transfused) or to MPC. For packed red-blood-cell, the incidence was 1:173 000 transfused units.

An antibody-mediated mechanism was established in 30 of the 50 cases (60%) with a complete immunological work-up. In these cases, HNA or HLA antibodies in at least one donor reacted with a cognate antigen of the recipient or alternatively the cross-match reaction was found positive between the plasma of a donor and the recipient cells using a microlymphocytotoxicity test. Data for 2010, the year on which prevention steps described above were taken, are yet not available.

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### Question 1

For transfusion purposes, in Germany, only the use of plasma from men and from women either without a history of pregnancy or who were tested negative for leucocyte antibodies is allowed [1]. There are no restrictions concerning the transfusion history of the donors.

### Question 2

Concerning the prevention of TRALI, there are currently no regulations in force for platelet concentrates in Germany. Most blood services apply the same regulations for the selection of platelet apheresis donors as for plasma donors (see answer to question 1). Reduction in plasma in platelet concentrates by platelet additive solutions is done by some blood services but not as a dedicated safety measure for the prevention of TRALI.

### Question 3

We and some other blood services and blood banks screen female donors with a history of pregnancy for leucocyte antibodies, that is, antibodies directed against HLA class I, class II and HNA. If the test result is positive, it is not allowed to transfuse plasma from these donors. In addition, donors with HNA-1a, 1b, 2 and 3a antibodies have to be excluded from further platelet and whole-blood donations [1].

### Question 4

We do not use other measures, and there are also not other ones recommended by the competent authority.

### Question 5

We use the techniques as recommended by the ISBT Working Party on Granulocyte Immunobiology [2], that is, for the detection of HNA antibodies the granulocyte immunofluorescence test and the granulocyte agglutination test; for the detection of HLA class I antibodies, the lymphocyte immunofluorescence test; and for the detection of HLA class II antibodies, a commercial ELISA. There are high-throughput assays under development based on microbead or ELISA techniques. However, their sensitivities will have to be adapted for transfusion needs.

### Question 6

Reporting frequencies for antibody-mediated TRALI were published by the Paul-Ehrlich-Institute for the years 2006/2007 as follows [3]: plasma (FFP): 1:66 000, platelet concentrates: 1:420 000 and red-blood-cells: 1:2 860 000. In the interval of 2006–2009, 78% of the reported TRALI cases (61/78) in Germany fulfilling the TRALI criteria of the International Haemovigilance Network were associated with donor leucocyte antibodies [1, 3–5]. The 14 TRALI fatalities (18%) occurred in TRALI cases with leucocyte antibodies, and none of the non-immune TRALI cases had a fatal outcome. Preventive measures such as described in question 1 became mandatory for German blood services from September 2009 on. In 2010 and until November 2011, the number of TRALI cases reported to the haemovigilance registry has been reduced to six, of which only two were due to leucocyte antibodies, one after the transfusion of red-blood-cells and the other after the transfusion of a platelet concentrate. According to a notification of the Paul-Ehrlich-Institute, no TRALI-related fatalities have been reported after 2009 and until November 2011. This indicates that the TRALI preventive measures were very successful as they have reduced significantly the number of TRALI events [1].

In all cases reported to the Blood Service West, the TRALI reporting and/or observing physicians have been directly contacted as recommended [6]. After introduction of the preventive measures in our blood service already in 2006, we have not observed any case of fatal or severe (requiring artificial ventilation) TRALI due to leucocyte antibodies any more.

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- (1) The Hong Kong Red Cross Blood Transfusion Service (HKRCBTS) uses only FFP derived from whole blood donated by males, irrespective of their transfusion history, for clinical transfusion.
- (2) Almost all our platelets supplied for clinical transfusion are whole-blood-derived and not restricted to donation by males. We do not reduce the amount of plasma in platelet concentrates, but plan to explore the technology for future application. However, we think that male donors (irrespective of history of transfusion) are preferable, but not exclusive, for apheresis platelet donation.
- (3) We do not screen blood donors for HLA or HNA antibodies.
- (4) We have commenced supplying pathogen reduction-treated FFP (methylene blue and white light) to paediatric patients for clinical transfusion since early this year for the purpose of enhancing blood safety. So far, the accumulated data were not sufficient for meaningful analysis. We are currently planning to study the production and supply of pathogen reduction-treated platelet concentrates for clinical use.
- (5) We do not screen blood donors for HLA or HNA antibodies.
- (6) In 2010, we had one confirmed TRALI case after 577 167 blood components issued. The incidence of definite TRALI in Hong Kong was therefore 0.17 per 100 000 components. That TRALI case was associated with platelet transfusion and anti-HLA antibodies were found in the donor plasma with specificity against the

recipient's HLA class I antigen. With this finding, we are exploring the feasibility to implement platelet additive solution to replace plasma in platelet concentrates to mitigate the risk.

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- (1) A small stock of FFP is produced in Ireland for use at a liver transplant unit and at a paediatric cardiac surgery unit. FFP is produced from male donors only; all donors are untransfused or have not been transfused since 1980. From October 2002, individuals who had previously received blood transfusions outside the Republic of Ireland were excluded from donating (whole) blood and apheresis in Ireland.

The alternative product used since March 2002 is solvent detergent (SD) plasma (Octaplas/Uniplas) produced in USA from plasma collected in the USA at IBTS inspected Centres from voluntary unpaid male or female donors (See response to Question 4 below). The use of SD plasma in Ireland was a measure to reduce the risk of transmission of vCJD by blood transfusion rather than a TRALI reduction measure.

- (2)
  - (a) Yes. Pooled buffy-coat platelet concentrate is resuspended in platelet additive solution (PAS) 210 ml (70%) with residual 90 ml male or female donor plasma (30%). Prior to buffy-coat platelet concentrate being resuspended in PAS, one of the four pooled donors had to be male and his plasma, not female plasma, was used for resuspension but there was always a small amount of residual female plasma in the suspension.  
 Apheresis platelets are currently suspended in c. 230 ml donor plasma. The latter will be changed to platelet additive solution in mid-2012 but will still contain residual 35% plasma (c. 80–90 ml) male or female plasma.
  - (b) No. Since October 2003, donor restriction<sup>#</sup> was introduced, that is, any new donor recruited to the apheresis panel must never have been pregnant and must never have been transfused (in Ireland or outside Ireland at any time). Women who were on the platelet donor panel prior to October 2003, who had not been implicated in a transfusion reaction with/without the

history of pregnancy, continue to donate for platelet concentrate. Currently, there are circa 120 such female donors (exact number will be known by end January 2012) still on apheresis panel recruited prior to October 2003. Our intention is to test these donors for HLA and antigranulocyte antibodies if they have ever been pregnant.

- (3)
- (a) No we do not screen. In November 2011, it was decided to commence screening female apheresis donors, who have ever been pregnant, for HLA class I and II antibodies and if negative, then for granulocyte antibodies as a TRALI risk reduction measure.
- (b) We will do so. Donors who are positive will be resigned from the apheresis panel (and advised to go back to the whole-blood panel).
- (4) Yes solvent detergent treatment as a virus inactivation measure for plasma introduced 2002.

There are no confirmed reports of TRALI from Octaplas/Uniplas, in spite of several million transfusions since 1991. Therefore, for whatever reason the SD process (or the pooling process) seems to completely eliminate the risk. So there is no gender specification for Uniplas/Octaplas.

No pathogen inactivation measure is used for FFP or platelet concentrate. The latter are subject to bacterial screening (BacT/alert).

- (5)
- (a) We do not routinely perform HLA antibody screening for platelet donors. Screening for HLA antibodies (only) is performed for NAITP, PTP, TRALI, refractoriness to platelet transfusions and transfusion reactions.
- (b) Technique = Fluorescent bead assay (Luminex)

(c) Yes. We intend to move towards screening female donors (*c.* 120) on current apheresis platelet panel, recruited prior to October 2003 who have ever been pregnant, for HLA class I and II & HNA (granulocyte) antibodies as a TRALI risk reduction measure.

- (6)
- (a) Yes See Table 1 below. Too few cases to determine effect but incidence remains low.
- (b) 75% due to anti-HLA antibodies.

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#### Question 1

Starting from 1 April 2011, FFP for clinical use is obtained in Italy from non-transfused men only.

#### Question 2

In 2010, we produced 4356 adult platelet doses. Of these, 4127 (94.7%), 212 (4.9%) and 17 (0.4%) were prepared using the buffy-coat method, the platelet-rich plasma technique and apheresis, respectively. All platelet products prepared in our blood transfusion service are resuspended in a commercial crystalloid platelet additive solution. The final storage media consist of approximately 30% plasma and 70% crystalloid solution.

**Table 1** Confirmed/possible TRALI

	RCC	Plasma (FFP)	Platelet
2001	3 Highly probable Positive XM (1) Positive donor ABs (1)	Positive Donor ABs (1)	0
2002 (SD plasma introduced)	1		Pooled buffy coat. Possible. Incomplete investigation.
2003 (Donor restriction <sup>#</sup> )	1		Apheresis. Highly probable. Positive Donor Abs. Fatal
2004	0		
2005	0		
2006	2 Possible. Positive Donor ABs (2)		
2007	0		
2008	1 Possible		
2009	0		
2010	0		
2011	0		
Total	8 5	1	2

### Question 3

We do not routinely screen male transfused donors and female donors reporting pregnancy for HLA and HNA antibodies. Of note, RBC also, in addition to the platelet products, are resuspended in crystalloid additive solution. Therefore, the only plasma-rich blood component for direct clinical use is FFP collected from untransfused male donors. Although we do not perform routine screening as reported above, we perform HLA and HNA antibody detection as part of the laboratory work-up in TRALI/suspected TRALI cases.

### Question 4

Pathogen reduction technologies that are currently commercially available for the treatment of plasma and platelet products are not routinely used in our blood transfusion service.

### Question 5

For HLA class I and II antibody detection, we use FLOW-PRA class I and II screening (One Lambda). For HNA antibody screening we use both granulocyte immunofluorescence test (GIFT) by flow cytometry and granulocyte agglutination test (GAT).

With regard to novel technologies, we are starting to evaluate the combination screening (HLA + HNA) with Labscreen Multi (OneLambda) but this kit, which is designed for Luminex, evaluates HNA-1a/-1b/-1c and HNA2 only, thus missing the other HNA specificities.

### Question 6

In a regional Italian study, recently performed in our country [1], we reported 10 TRALI/possible TRALI cases after the transfusion of approximately 640 000 blood components. In particular, 6, 4 and 0 TRALI/possible TRALI cases occurred after the transfusion of approximately 490 000 RBC (frequency = 1:82 000), 90 000 FFP (1:22 500) and 60 000 adult platelet doses, respectively. The frequencies of TRALI/possible TRALI cases detected in our region were similar to those reported in the recent literature [2, 3], except for TRALI associated with platelet transfusion, which was not found in our series. This may be due to the prevalent use of multiple donor platelets suspended in crystalloid solution in the blood centres of our region, which decreases the potential impact of platelets resuspended in plasma from single or multiple donors with high-titre HLA and/or HNA antibodies.

As far as the antibody specificities detected in our 10 cases, seven (70%) were immuno-mediated TRALI/possible TRALI cases. In four cases, we found HLA antibodies and the cognate antigens in the recipients. The principal HLA antibody specificity was related to class II antigens; in particular, in three cases, we detected anti-HLA-DR5 specificity, which represents an antigen of the DR52 broad cluster,

thus confirming the important role of anti-HLA class II alloantibodies in the development of TRALI [4]. We also observed three cases in which HNA antibodies were implicated (30%), but no particular antigen specificity was identified.

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H. Okazaki & K. Tadokoro

### Question 1

First of all, transfused persons are currently excluded from donation regardless of their gender in Japan. We have three different plasma products, namely FFP-LR1 prepared from 200-ml whole blood, FFP-LR2 prepared from 400-ml whole blood, and FFP-LR Ap prepared by plasmapheresis (LR stands for leucocyte-reduced). Among these three plasma products, FFP-LR2 is the most common product, which accounts for 75% of the total FFP supply in Japan; in addition, 80% of the FFP-LR2 supply has already been prepared from male donors. We first attempted to prepare FFP-LR2 only from male donors in July 2010. We also updated the

computer system in April 2011, which allows the staff members in the production department to sort male and female blood donations automatically. In 1 month after system implementation nationwide, we successfully increased the production rate of FFP-LR2 from male plasma up to more than 98%.

Regarding FFP-LR1 derived from 200-ml whole blood, which accounts for about 7% of the total FFP supply, the percentage of female donors is approximately 75%. One of the core blood centres has successfully implemented the same strategy for producing FFP-LR1 as that for producing FFP-LR2, but other core blood centres have not implemented such a strategy yet. Moreover, we continuously increase the rate of 400-ml whole-blood donation instead of 200-ml whole-blood donation, which will subsequently raise the percentage of the supply of FFP prepared from male donors.

Regarding FFP-LR Ap prepared by plasmapheresis which accounts for about 18% of the total FFP supply, the percentage of female donors for this product is approximately 50%. However, this relatively high percentage of female donors for this product did not enforce us to implement male-only plasma in apheresis plasma. HLA antibody screening will be a possible way to reduce the risk of TRALI caused by FFP-LR Ap.

#### *Question 2*

We do not replace plasma in platelet concentrates with a platelet additive solution. As noted above, we do not use male-only donors for apheresis platelets.

#### *Question 3*

We are now preparing an HLA antibody screening test for female donors regardless of their pregnancy history, because presently our questionnaire does not have any items regarding the pregnancy history of female donors.

#### *Question 4*

Once donors are proved to be associated with TRALI cases and to have antileucocyte antibodies, their donations are not used for transfusion but only for research. We do not have any data about the effectiveness of these preventive measures.

#### *Question 5*

We have not performed any screening yet for these antibodies; however, we are now evaluating the usefulness of ELISA for HLA antibody screening. Currently, we are not considering HNA antibody screening.

#### *Question 6*

In the last 7 years from 2004 to 2010, 275 cases of TRALI (172 cases of TRALI and 103 cases of possible TRALI) were

reported to the Japanese Red Cross. The incidence of TRALI was approximately 1 in 130 000. We have just recently started the male-only-donor strategy for 400-ml whole-blood-derived plasma (FFP-LR2), and we will see the results in the next 2 or 3 years.

Among the above-mentioned TRALI cases, HLA and/or HNA antibodies were detected in 35% of the cases (97 cases), which was associated with at least one donor. There were 25 cases in which the cognate antigens were detected (positive in the cross-match test) in patients (20 cases of TRALI and five cases of possible TRALI) out of 44 cases in which we were able to obtain blood samples from patients.

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#### *Question 1*

We do not obtain only male blood for FFP.

#### *Question 2*

We do not reduce the amount of plasma in platelet concentrates nor replace it with platelet additive solution. Of the fourth blood banks participating in this inquest, only one \* non-transfused male donor is used for apheresis platelets.

#### *Question 3*

Not one of the blood banks inquested, screen donors looking for HLA or HNA antibodies.

#### *Question 4*

We do not use other measures that might prevent TRALI.

#### *Question 5*

We do not screen donors for HLA and HNA antibodies. Actually we do lymphocytotoxicity test for antileucocyte antibodies in serum as a diagnostic tool in blood transfusion febrile reactions, and flow cytometry for transplant purposes. We do not foresee technical developments in this field in our laboratory routine work.

#### *Question 6*

We do not know the incidence of TRALI in Mexico.



Information obtained from the participating blood banks physicians:

National Institute of Cancerology. Sergio Arturo Sánchez-Guerrero MD: obtains 8887 blood units, 12 263 components, 1717 apheresis platelets annually. Two cases TRALI suspected clinically.

\*Transfusion Medicine and blood bank, Medica Sur hospital. Héctor A. Baptista-González MD: obtains 4256 blood units; 3762 FFP units, 698 Apheresis platelets annually. One case TRALI clinically suspected.

Central blood bank of Siglo XXI National Medical Center of Mexican Social Security. Carlos Martínez – Murillo MD: obtains 60 000 blood units, 210 000 blood components annually. Never seen TRALI cases.

Central blood bank of La Raza National Medical Center of Mexican Social Security. Angel Guerra Márquez MD: obtains 75 876 blood units, 49 768 FFP units, 56 760 PC annually. One case TRALI clinically suspected.

In my opinion is very difficult ARDS from TRALI diagnostic distinction [1]. In our country, diagnosis, as it has been reported, is very rare, despite the incidence of natality ( $17.5 \times 1000$ ), and the percentage of female blood donors is near 25% [2]. TRALI clinical diagnosis should be more frequent; however, it is not.

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### Question 1

In the Netherlands, approximately 670 000 blood components, representing 530 000 units red-blood-cell concentrates (RBC), 57 000 platelet products and 83 000 units quarantine fresh frozen plasma or FFP (as equivalents of circa 300 ml), are yearly supplied to the hospitals for individual patient use [1]. Since October 2006, quarantine FFPs are obtained solely by plasmapheresis from untransfused male donors, and from July 2007, such units have exclusively been delivered to hospitals.

### Question 2

Yearly over 57 000 platelet products (57 346/2010) are used in our country. The majority is prepared from five pooled buffy coats from random donors. After preparation, circa 20% is preserved in a mixture of 30% plasma and 70% platelet additive solution PAS. The majority of pooled platelet products are preserved in plasma derived from one donor whose platelets are part of the buffy-coat pool. Since November 2009, the unit of plasma used to store the platelet product is selected from a male non-transfused donor, contributing to the buffy-coat pool. A minority (ca 7500) of platelet products required for pre-term born infants and/or for alloimmunized recipients consist of single-donor apheresis platelets. In case of HLA/HPA-matched platelets, all donors, including multiparous women, are allowed to donate platelets stored in plasma. We considered this approach justified because HLA class I antibodies will not recognize foreign HLA antigens in the recipient, HLA class II antibodies pose a reduced risk because of linkage with the HLA-B locus and granulocyte-specific antibodies are rare. In a large cohort of alloexposed Dutch blood donors, the prevalence of granulocyte-reactive antibodies was 3% as compared to 2% in never alloexposed donors [2].

### Question 3

We do not routinely screen for leucocyte-reactive antibodies with the intent of donor exclusion. The current untransfused male policy to donate plasma either for Q-FFP or for the preservation of platelet concentrates from pooled buffy coats in plasma causes no major constraint on logistics and available blood supply.

#### Question 4

It is unlikely that pathogen reduction methods of FFP and/or platelets will reduce alloimmune or innate immune causes of TRALI. Pooling of plasma from multiple donors neutralizes and subsequently removes leucocyte-antibody complexes and reduces TRALI as has been shown by reports from countries using pooled solvent detergent (SD) plasma. SD plasma is currently considered for the Netherlands but not to further reduce TRALI. Other preventive measures such as poststorage and pretransfusion washing of RBC and platelets, removing antibodies and – during storage accumulated inflammatory mediators – needs more research before considering such approach [3].

#### Question 5

As mentioned, we do not screen for the purpose of donor exclusion. Currently, we accept the risk that incidentally TRALI has been demonstrated after 10–20 ml of plasma, present in RBC or a pooled platelet product, that contained multiple leucocyte-reactive antibodies including antibodies against the recipient [4]. We are concerned that if it would be decided to screen the whole donor population, the choice of technique(s) will be based on feasibility for high throughput, robustness and costs rather than on clinical relevance and will definitely lead to unnecessary donor loss. It is widely acknowledged from antibody prevalence studies in the donor population and look-back studies that the majority of serologically detected leucocyte-reactive antibodies do not cause TRALI. As long as the mechanism of TRALI is incompletely unravelled, a reliable functional instead of serological assay is still a holy grail, although a few antibody specificities (e.g. anti-HLA-A2) seem on empirical data to emerge as more clinically relevant.

#### Question 6

Dutch Hemovigilance registration started in 2002 with 90% of hospitals participating. Until 2005, the reported TRALI cases increased, presumably due to teaching and growing awareness of clinicians regarding this transfusion complication. The incidence of TRALI, as defined by the Canadian Consensus Conference in 2004, was investigated for the 8-year period 2002–2009 as a before and after study. After the 'male-only' measure in 2007, total TRALI decreased by one-third (95% CI 0.09–0.51), thought to reflect the contribution of plasma to the occurrence of TRALI [5]. Virtually, all cases of TRALI after transfusion of plasma disappeared. Of 12 reported TRALI cases in 2010, eight were associated with RBC transfusions, four with a mixture of pooled platelet transfusions combined with other blood products and only one case of TRALI was associated with plasma transfusion [1].

A study investigating the imputability of donor anti-recipient leucocyte-reactive antibodies identified these

antibodies in only 48% of retrospectively analysed cases [6]. Although this does not prove causality, it is strongly suggested that the majority of these TRALI was indeed caused by these antibodies.

In conclusion, it is obvious that leucocyte-reactive antibodies against recipient leucocytes explain less than half of TRALI cases. Consequently, sensitivity and specificity of leucocyte-alloreactive antibodies, irrespective of the detection technique used, both are weak. It is an urgent challenge to identify the alternative causes of TRALI and patients at risk before preliminary and expensive measures, associated with donor rejection, are preliminary implemented.

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#### Question 1

In February 2008, the New Zealand Blood Service introduced a requirement restricting the production of FFP to male donors with no history of transfusion.

The single exception is the preparation of IgA-deficient FFP for transfusion which, due to the low incidence of IgA-deficient donors, may be manufactured from female donor plasma.

**Question 2**

Validation is currently underway for the manufacture of both buffy-coat pooled and plateletpheresis platelet concentrates using platelet additive solution as a replacement for plasma. At present, approximately 28% of apheresis platelet donations and 74% of pooled platelets are prepared in this manner. The remainder are suspended in plasma. Once validation is completed, all platelets concentrates other than those manufactured for neonatal patients (< 6 months of age) will be suspended in 65% platelet additive solution/35% plasma.

Apheresis platelets are currently collected from both female and male donors irrespective of transfusion history.

**Question 3**

The New Zealand Blood Service is in the process of introducing further TRALI risk reduction measures that will be implemented in mid-2012. All female plateletpheresis donors with a history of pregnancy will be screened for HLA class I and class II antibodies; if the result is positive, they will be excluded for plasma-rich blood components. As granulocyte antibody screening is not currently available within New Zealand, HNA testing will not be required as part of the risk management protocol until such a time as NZBS develops the internal capacity to screen.

**Question 4**

None at present.

**Question 5**

The National Tissue Typing Laboratory NZBS uses One Lambda Luminex class I and class II (Canoga Park, Los Angeles, CA, USA) beads to screen for HLA class I and class II antibodies. An in-house study was undertaken to determine the appropriate normalized background ratio cut-off to identify positive results, based on the method described by Triulzi *et al.* [1] Women currently account for 57% of the plateletpheresis panel. Results of initial testing suggest that this will result in the loss of 23% of these donors.

For donor screening, the most important technical development in the near future would be the availability of high-throughput methods for simultaneous HLA and HNA antibody screening.

**Question 6**

The NZBS operates the National Haemovigilance Office which receives reports of transfusion-related adverse events from Blood Bank Scientists or Transfusion Nurse Specialists, from the 20 District Health Boards (DHBs) located throughout New Zealand. Reporting to the programme is voluntary. A Haemovigilance Annual Report is issued and is available on the NZBS website ([www.nzblood.co.nz](http://www.nzblood.co.nz)).

Table 1 summarizes the reported TRALI cases with imputability categorized from possible to certain for the previous 5 years [2].

The difference in the rate of TRALI cases pre- and postintroduction of male-donor-only FFP was statistically significant ( $P < 0.02$ ).

In 2008, a retrospective review of investigations performed by the National Tissue Typing Laboratory on TRALI cases from June 2004 to June 2007 was undertaken [3]. Seventeen cases were investigated over the 3-year period. A total of 67 donors were tested, and 19 (43%) had positive HLA antibody screens. Fifteen of the seventeen cases (88%) had donors with positive HLA antibody screens.

In nine of the seventeen cases (53%), there was either a positive cross-match or the specificity of the antibody was directed against the antigen present in the recipient.

HNA antibody testing was performed by the Australian Red Cross Blood Service Platelet and Granulocyte Reference Laboratory in Brisbane. No neutrophil-specific antibodies were demonstrated.

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**Table 1** Reported TRALI cases

Year	Reported TRALI cases	Components transfused/ annum	Reported TRALI cases/100 000 components transfused	Implicated components
2006	10	152 259	6.57	N/A
2007	9	153 136	5.88	FFP = 6; Apheresis Platelet = 1 RBC = 1
2008 <sup>a</sup>	4	156 188	2.56	Apheresis Platelet = 1 Platelet pool = 1 RBC = 1
2009	1	160 293	0.62	Platelet pool = 1
2010 <sup>b</sup>	3	159 597	1.88	Platelet pool = 1 RBC = 2

<sup>a</sup>Male-donor-only FFP introduced February.

<sup>b</sup>Unpublished data.

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### Question 1

At present we do not; we are, however, seriously considering such possibility.

### Question 2

The quality of platelet concentrates (PCs) stored in additive solution (SSP+) was subjected to studies at the Institute of Hematology and Transfusion Medicine in 2007. We selected the new-generation solution (PAS III M) which apart from sodium chloride, citrate, acetate and phosphate also contains magnesium and potassium salts that have been proved to significantly decrease the platelet aggregation. The *in vitro* quality of platelets was maintained up to 7 days if they were stored in a mixture of 80% SSP+ and 20% plasma. It was impossible to extend the storage time beyond 7 days due to high (statistically significant) glucose consumption; pH remained constant during the whole storage period. We also found that PCs with  $3.0 \times 10^{11}$  platelet count should be stored in approximately 250 ml of the mixture.

At present, the SSP+ solution produced by Macopharma is used in seven of the 21 regional blood centres: Lublin, Kielce, Łódź, Wrocław, Warszawa, Poznań and Białystok.

In three of these centres, PCs are prepared with SSP+ using the OrbiSac Blood Component Processing System (TerumoBCT). Routine practice involves pooling 5–6 buffy coats and suspending them in a mixture of 65% SSP+ and 35% plasma. The other four centres use SSP+ both for storage and for the preparation of washed PCs. These procedures are used as preventive measures against transfusion reactions.

### Question 3

We do not screen donors for HLA class I and class II and HNA antibodies. However, all available donors of blood components transfused to patients with observed reactions, such as sudden onset of respiratory distress, with dyspnea and tachypnea and typically acute hypoxemia, fever, tachycardia and/or hypertension, are tested for antileucocyte antibodies (anti-HLA and anti-HNA).

If such antibodies are detected, the donor is deferred from donation of blood for clinical use.

### Question 4

Pathogen inactivation technology (Mirasol PRT, TerumoBCT) was introduced in 2009 in one regional blood centre located in Warsaw where six illuminators were installed (two for PC and four for FFP). By the end of November 2011, 25 illuminators were installed in nine regional blood centres (three for PC and 22 for FFP). This technology is in routine use only in Warsaw Regional Blood Center, where approximately 13 000 therapeutic PC doses (65% apheresis PCs) and 63 000 units of FFP are produced annually. In the period July 2009–October 2011, approximately 14 500 therapeutic doses of Mirasol-treated PCs (50% apheresis PC) were transfused. No transfusion reactions were reported. In the Institute of Hematology and Transfusion Medicine, more than 1500 Mirasol-treated PC therapeutic doses were transfused to 258 patients with different haematological disorders. No TRALI cases were observed. In the period January 2010–October 2011, approximately 33 000 units of Mirasol-treated FFP was transfused. Again, no transfusion reactions were reported. In our hospital, 15 patients were administered 555 units of Mirasol-treated FFP. One of these patients was transfused with 459 units of Mirasol-treated FFP and no transfusion reactions were observed. In future, all fresh frozen plasma for clinical use will be subjected to inactivation.

### Question 5

TRALI diagnostics is performed according to the recommendations of ISBT Working Group on Granulocyte Immunobiology (Bierling *et al.*, *Vox Sang* 2009). We routinely perform enzyme-linked immunosorbent assay (ELISA) and lymphocytotoxicity test (LCT) for anti-HLA

and granulocyte agglutination test (GAT), granulocyte immunofluorescence test (GIFT) and monoclonal antibody immobilization of granulocyte antigens (MAIGA) for anti-HNA detection. The process of implementation of the Luminex® technology for HLA antibody detection is ongoing.

As regards the screening programme for TRALI prevention, we plan to perform the preliminary study of Polish blood donors with the above-mentioned methods including RQ PCR for HNA3 genotyping followed by anti-HNA3a testing. Based on the results of the study, a screening strategy will be suggested, preceded by cost estimation and its impact on donor loss. The outcome of our analysis will be forwarded to the Ministry of Health for decision.

#### Question 6

TRALI incidence is analysed in Poland on regular basis. The diagnostic centre is localized in the Department of Immunohematology of the Institute of Hematology and Transfusion Medicine. Clinical observations and laboratory data have been published in several papers, and they are regularly presented at congresses and lectures. The data for 2001–2005 as summarized by Zupanska *et al.* showed 16 TRALI cases and 28 possible TRALI cases; anti-HLA/anti-granulocyte antibodies were detected in 68.2% of all cases. In the 2006–2011 period, we diagnosed 13 TRALI cases and 15 possible TRALI cases (0 – in 2006; 6 – in 2007; 5 – in 2008; 10 – in 2009; 5 – in 2010; 2 – in 2011); anti-HLA/antigranulocyte antibodies were detected in 64.3% of cases.

#### Summary

In Poland, the incidence rate for reported TRALI or possible TRALI cases varies from 0 to 10. Although an educational programme for TRALI diagnostics (less severe TRALI included) has been implemented (which should imply that the number of reported TRALI cases will increase), what has in fact been observed was a slight decrease in the incidence rate (8.8 cases annually in 2001–2005 and 4.7 annually in the later periods). This tendency might be attributed to the implementation on a larger scale of leucoreduction and inactivation techniques. It is worth mentioning that in 2011 in Poland, we observed only two cases of TRALI, although the number of transfusions was slightly higher than in the previous years.

As regards antibody detection, we can also expect improvement as result of the implementation of additional/methods (ELISA in 2005; Flow PRA in 2007). However, the frequency of immunological TRALI is similar during the period 2001–2005 and later.

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E. Zhiburt

- (1) Yes, we in Pirogov Center use FFP from male donors since 9 July 2010. Since 2012, we will use only non-transfused men as donors for FFP.
- (2) No, we are just planning to use SSP+ additive solution for platelets.
- (3) No, we do not do such screening.
- (4) Pathogen-inactivated plasma and platelets are widely used in Russia without any connection with TRALI prevention.
- (5) No, we do not screen for HLA and HPA antibodies.
- (6) We do not have clear data of incidence of TRALI in our country. Probably sometimes TRALI is mistakenly considered as pneumonia [1].

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E. Castro

#### Question 1

The jurisdiction over blood transfusion in Spain has been transferred to the 17 Autonomous Communities. Although there is state legislation emanating from European Directives that regulates this matter, aspects such as those debated in this forum are not regulated, and therefore, different practices can be found throughout Spain. In these cases, procedures are determined by the state-of-the-art and publications with a high level of scientific evidence [1, 2].

In order to reflect the current situation in Spain, a questionnaire was sent to the directors of all the Spanish blood transfusion centres and replies have been received from the following: Galicia, Asturias, Cantabria, Basque Country,



Navarra, Aragon, Valencia, Andalusia, Castile and La Mancha, Balearic Islands, Madrid and the Red Cross Blood Transfusion Centre in Madrid.

The majority of Community Transfusion Centres (Galicia, Asturias, Cantabria, Basque Country, Aragon, Balearic Islands and Spanish Red Cross in Madrid) use plasma exclusively from male donors who have never been transfused. The Blood Transfusion Centres in the Community of Madrid, Castile and La Mancha, Navarra and Andalusia use plasma from donors of both genders regardless of the number of previous transfusions.

#### Question 2

In Spain, the majority of platelets are obtained in additive solution; this applies to platelet pools derived from whole blood as well as those obtained through apheresis.

The only centre that performs HLA antibody screening of apheresis donors is in Cantabria, which carries out the test on female plasma and platelet donors, and only admits those with negative results.

The Cadiz Blood Transfusion Centre produces both pooled platelets in additive solution and apheresis platelets suspended in plasma (that accounts for only 2% of the total therapeutic doses of platelets produced) and does not select donors by gender.

#### Question 3

None of the centres surveyed performed this type of screening.

#### Question 4

With the exception of blood transfusion centres in Andalusia and Navarra, which use quarantined plasma regardless of the gender of the donors, and the transfusion centres in Cantabria, the Basque Country and the Balearic Islands, which use quarantined plasma from untransfused males, all the remaining transfusion centres surveyed prepare plasma that is inactivated through different systems. The majority use the methylene blue system, and only the Red Cross Transfusion Centre in Madrid uses the inactivation system based on amotosalen and ultraviolet A light (Intercept™ for plasma; Cerus Corporation, Concord, CA, USA).

With respect to platelet products, various centres use inactivation systems: Galicia, Asturias, Balearic Islands and the Red Cross Transfusion Centre in Madrid on 100% of their products, and the Castile and La Mancha Transfusion Centre on 18% of the same. All these use the system based on amotosalen and ultraviolet A light (Intercept™ for platelets; Cerus Corp.). The Navarra Transfusion Centre uses the pathogen inactivation system based on riboflavin and ultraviolet light (Mirasol; Caridian BCT, Lakewood, CO, USA; Mirasol PRT, Terumo BCT).

#### Question 5

Although none of the transfusion centres carries out HLA or HNA antibody screening of donors, three centres have these techniques available and use them either for HLA screening of female apheresis donors or in the retrospective study of donors implicated in a case of TRALI.

These centres are as follows: the Transfusion Centre of Cantabria, which has used ELISA techniques for class I anti-HLA antibodies and currently uses the Luminex system; the Transfusion Centre of Valencia, which uses a lymphocytotoxicity technique for HLA and a flow cytometry technique for HNA; and the Transfusion Centre of Galicia, which performs HLA antibody screening using lymphocytotoxicity and ELISA techniques.

#### Question 6

The Spanish Hemovigilance system collects data related to adverse reactions experienced during blood transfusions throughout Spain. The last report, published in 2011, reflects the data collected during 2010.

A total of 8 070 015 units of labile blood components were delivered by Spanish blood centres in 2007–2010: 6 300 331 RBC units, 701 526 therapeutic doses of platelets (combined pooled buffy-coat platelets and apheresis platelets) and 227 268 l of plasma, which equals about 840 890 units of whole-blood-derived fresh frozen plasma units. During this period, 77 cases of TRALI with imputability levels equal or higher than 2 were reported to the Spanish Hemovigilance system, divided as follows: 30 related to RBC, 14 to platelets, 13 to plasma and 17 to multicomponent.

Combining both series of data, the general risk for documented TRALI was 1:104 805 and the per component risk during the same period was 1:210 011 for RBC; 1:50 109 for platelets and 1:64 683 for plasma. These data are similar to those published by other haemovigilance systems such as the British SHOT and French systems [3].

With respect to knowing whether the preventive measures that have been adopted are effective, given that these measures have only been implemented by the majority of centres during the last 2–3 years, at the moment it is difficult to determine the efficacy of the same [1].

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L. Puig Rovira & E. Muñiz-Díaz

### Question 1

By the end of 2008, we fully implemented the policy of using plasma from male donors for transfusion. However, the transfusion antecedents in plasma male donors are not considered.

### Question 2

Platelet concentrates obtained from whole-blood donations are prepared with the Atrius 3C system, which produces an intermediate product called intermediate platelet unit (IPU), containing platelets from whole blood suspended in approximately 20 mL of plasma. The final platelet pool consists of four IPUs suspended in 200 mL of SSP solution and filtered to produce a pool of leuco-reduced platelets.

Of 33 435 platelet concentrates transfused in our region in 2010, only 704 were obtained from apheresis donation. In general, no female donors or no donors with transfusion antecedents were excluded from platelet apheresis donation.

### Question 3

No, we do not screen our donors for leucocyte antibodies.

However, all suspected TRALI cases are studied in our laboratory, and when a donor implicated in a TRALI case is found to have HNA, HLA class I or class II antibodies with a specificity directed against an antigen in the recipient's WBCs, the donor is deferred from donation. Even if recipient leucocytes cannot be tested, we prefer to defer the donor from donation. The exception to this policy occurs in the case of donors in whom HLA class I antibodies are the only antibodies detected and appropriate samples from the patient are unavailable.

### Question 4

No additional measures have been adopted in our Blood Transfusion Centre.

### Question 5

In the studies of TRALI, we follow the 'Recommendations of the Granulocyte Working Party on Granulocyte Immunobiology of the ISBT'. A combination of the granulocyte immunofluorescence test (GIFT) and the granulocyte agglutination test (GAG) is employed for HNA antibody detection, and all positive results are confirmed with the monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA) assay. HLA class I antibodies are investigated with a lymphocytotoxicity test and with an ELISA technique. HLA class II antibodies are also studied with an ELISA technique. HNA and HLA typing of the recipient and the donors are performed when indicated. Where possible when a donor leucocyte antibody is detected, a cross-match between donor plasma and recipient leucocytes is performed, using the GIFT, the GAT and the MAIGA tests. In the absence of cells for cross-matching, leucocyte typing of the recipient is performed for the cognate antigens recognized by the detected leucocyte antibodies.

If the donors for leucocyte antibodies were screened, the techniques employed would be the aforementioned ones.

### Question 6

In our region, Catalonia, with a population of seven million, 326 559 blood components were transfused in 2010. The first annual Haemovigilance report was elaborated in 2003, and during the last 8 years, 55 cases of TRALI were reported to our register. In the middle of 2007, we decided to progressively implement the measure of not transfusing plasma from female donors, and this was fully implemented by the end of 2008. In 2003–2008, 48 TRALI cases were reported, 15 (31%) associated with the plasma transfusion, 9 (19%) with platelets, 18 (37%) with red cells and 6 (13%) with multicomponents. In the last 2 years (2009–2010) when no plasma from female donors was transfused, seven cases of TRALI were reported, 6 (86%) associated with the transfusion of red cells and one (14%) with multicomponents. Consequently, none of the TRALI cases reported was associated with plasma transfusion. Moreover, the incidence of TRALI fell from 1:43 000 transfusions in the first period to 1:96 000 in the last one.

The investigation of leucocyte antibodies has been systematically performed since 2006. Since then, 34 cases of suspected TRALI have been studied, obtaining positive results in 16 of them. All donors in whom leucocyte antibodies were identified were female donors. The unequivocal relationship between the antibodies found in the donor and the recipient cells was only demonstrated in three cases in which anti-HNA-3a, anti-HLA class I (A3) and anti-HLA class II antibodies were respectively identified. It was not possible to obtain a new sample from the patient to perform a cross-match and/or genotyping in the remaining cases.

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#### Question 1

The Swedish healthcare system is regionalized and in 6/7 of the university hospital regions only male plasma is used, although not exclusively from non-transfused male donors. In one region, also AB screen negative female plasma is used.

#### Question 2

At most of the hospitals, the amount of plasma is reduced with platelet additive solutions, especially for multiple donor platelets concentrates produced by the buffy-coat method. Apheresis donors are usually of both sexes but newly recruited donors are preferentially male.

#### Question 3

Donors who have received transfusions and/or have been pregnant are not screened for HLA or HNA antibodies.

In 1/7 regions, female AB donors are screened for HLA and HNA antibodies and excluded if found to be positive.

#### Question 4

In 2/7 regions, pathogen-inactivated platelets are used (Intercept) primarily for reducing the risk of bacterial contamination and not for preventing TRALI. In one region, pathogen-inactivated plasma (Octaplas) is increasingly used by some departments. It is yet too early to evaluate the efficacy of these measures.

#### Question 5

Granulocyte agglutination test (GAT), HLA class I and class II ELISA screening, MLC and the Luminex system for HLA antibodies are used. In future, important technical development is expected.

#### Question 6

The frequency of TRALI reactions reported to the Swedish haemovigilance system (BIS) has varied between 7.2% and 19% of all serious transfusion reactions in the years 2004–2007 (6–11 reports per year). Later, when only male plasma gradually started to be used, the frequency of TRALI decreased to 4.8% (four cases in a total of 84 reports) in 2009 and 3.1% (three cases in 96 reports) in 2010.

From 2010, also mild TRALI is reported to the national haemovigilance system as a separate type of adverse transfusion reaction. Three mild TRALI cases were reported in 2010.

Thus, in the past 7 years, a total of 51 TRALI and three mild TRALI cases were reported. Unfortunately, antibody investigations have been performed in only 29 cases. In 22/29 (75.9%) of these cases, HLA and/or HNA antibodies were found in the donor or occasionally in the patient. However, the percentage of TRALI cases due to antibodies could have been different if all cases had been tested.

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B. M. Frey

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#### Question 1

Since 2004, all donors having received blood transfusion after 1980 are permanently deferred from blood donation in Switzerland. Since 1 January 2007 Swiss Blood Transfusion Services (BTS) provide only FFP for transfusion which was manufactured from plasma donated exclusively by non-transfused males or by anti-HLA/HNA negative tested donors [1]. Recovered plasma that does not fulfil this requirement is used for fractionation only.

#### Question 2

Currently, all Swiss BTS manufacture platelet concentrates in additive solutions (Intersol<sup>®</sup>, Fenwal or SSP+, MacoPharma). By mid of 2011, the Swiss BTS have implemented the pathogen reduction procedure Intercept<sup>®</sup>, which requires partial replacement of donor's plasma. The residual donor plasma content in platelet concentrates ranges from 32% to 47%, according to the guardband criteria by CERUS. In order to guarantee sufficient supply of platelet concentrates, male and female donors are accepted for platelet apheresis donations, regardless of pregnancy history.

### Questions 3

At Zürich BTS, we are in the process of screening all platelet apheresis donors for anti-HLA I/II antibodies by ELISA regardless of pregnancy or transfusion history (see Question 5). So far, we found 5.8% of 1200 tested donors positive for anti-HLA class I and/or anti-HLA class II antibodies. However, there is a clear sex and pregnancy association of positive ELISA findings: mainly females (80%) show positive ELISA test results and most of them (81%) have been pregnant in the past. Due to supply restriction, it is not possible yet, to exclude anti-HLA I/II positive donors from donating apheresis platelet concentrates.

### Question 4

The main measure to prevent TRALI complication in Switzerland is the general use of FFP from non-transfused males or from anti-HLA I/II/HNA negative tested female donors [1]. Alternatively, solvent-detergent treated FFP (Octaplas®) which is less prone for TRALI complications is applied.

### Question 5

At the Zurich BTS, we performed anti-HLA class I, -class II, -TPA (anti-HLA I/II/TPA) ELISA screening using Quick-Screen®, PAK®2-LE and B-Screen® by GTi Diagnostics, Waukesha, WI, USA. These are sandwich ELISA tests with solid phase linked antigens to detect HLA-class specific and platelet specific antibodies in donor's serum. However, we have not yet decided on the exclusion criteria of ELISA positive donors. In the light of augmenting demand on platelet concentrates and the still ongoing controversy on clinical significance of anti-HLA I/II positive ELISA test results, we are reluctant to exclude platelet donors from donation exclusively based on laboratory findings.

### Question 6

According to the annual report of Swiss hemovigilance surveillance (<http://www.swissmedic.ch/marktueberwachung/00159/00160/00437/index.html?lang=de>) by Swissmedic, there is a decrease in frequency of TRALI complications as reported to the authority starting from 2008 (TRALI reports of all submitted reports, 2008: 5.5‰, 2009: 0‰, 2010: 1.5‰. In contrast 2007: 7.4‰, 2006: 12.6‰, 2005: 4.8‰, 2004: 8.6‰). However, the numbers are small although the reporting system is mandatory. The difference in the frequency of TRALI reports in the two periods may not be statistically significant and underreporting is likely/possible, which may result in reporting frequencies not completely reflecting the clinical situation in general.

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### Question 1

NHSBT uses exclusively male donors for FFP production from whole-blood and apheresis plasma donations. Preferential use of male donors for FFP production was introduced in late 2003. Currently, 100% of issued FFP, cryo-depleted plasma and cryoprecipitate issues has been produced from male donors, and this is under computer control.

Individuals who have been transfused since 1 January 1980 have been excluded from blood and component donation since April 2004 as a vCJD risk reduction measure. Donors who have been transfused before this time are not excluded. We do not systematically exclude eligible transfused male donors from FFP production.

### Question 2

We do not replace plasma in platelet pools or apheresis platelets as a TRALI risk reduction measure but ensure that 100% of donors who contribute the resuspending plasma to buffy-coat-derived platelet pools are males. Again, this is under computer control. We do not use exclusively non-transfused males but exclude all donors who have been transfused since 1980. Twenty per cent of our platelet production is from pools.

We have been instructed to produce 80% of platelet units by apheresis as a vCJD risk reduction method; it is not feasible to use male donors only to achieve this. Recruitment, however, is targeted towards males, and currently, 93% of apheresis platelet donors are males.

### Question 3

All female platelet apheresis donor recruits are screened for HLA class I and class II antibodies and if negative are also screened for granulocyte-specific antibodies. They are only eligible to be accepted if they are found to be negative for both. This applies to all female recruits whether or not they have been pregnant or transfused before 1980. Acceptance



of new female apheresis donors who had not been screened in this way ceased in July 2008.

The same screening has also been introduced recently (3 October 2011) for female platelet apheresis donors who began to donate platelets before screening of female recruits had become an acceptance requirement.

If HLA antibody screening tests are positive, donors are not eligible to donate platelets by apheresis but are eligible to continue as blood donors. Red cells are resuspended in optimal additive solution, and female plasma will not be used for FFP, cryoprecipitate or to suspend platelet pools. If donors test positive for IgG granulocyte-specific antibodies, they are permanently excluded from donating any blood component for clinical use.

Male donors who have been transfused (before 1980) are not screened for HLA or granulocyte-specific antibodies.

#### Question 4

FFP and cryoprecipitate for neonatal and paediatric use are manufactured by NHSBT from non-UK-derived whole blood or apheresis plasma, which is then treated with methylene blue as a viral inactivation method. SHOT has not received any report implicating either of these components in TRALI.

Pooled solvent detergent-treated FFP (Octaplas) is recommended in the UK for plasma exchange procedures in thrombotic thrombocytopenic purpura and is used by some hospitals for other clinical indications. SHOT has not received any reports of TRALI due to this product.

#### Question 5

Donors are screened for HLA class I and class II antibodies using One lambda™ LSM 12 assay (One Lambda Inc., Canoga Park, CA, USA). This is a solid-phase assay using HLA antigens attached to beads and a Luminex platform. If HLA antibody screening results are negative, donors are further screened for granulocyte-specific IgG and IgM antibodies using granulocyte and lymphocyte immunofluorescence tests (GIFT/LIFT) and the granulocyte chemiluminescence test (GCLT) with a granulocyte panel that covers HNA-1a, 1b, 1c, 2a, 3a, 3b, 4a, 4b, 5a and 5b.

With regard to future technical developments, a commercial company has developed a combined test kit capable of screening for antibodies directed towards HLA class I and class II, HNA-1a, 1b, 1c; HNA-2a; HNA-4a and MICA and MICB tested for on a Luminex platform. Currently, this does not include HNA-3 antigen but the gene for this has been sequenced, and it is likely that future kits will incorporate this. These are not yet commercially available. We are also interested in the potential use of cell lines expressing high levels of HNA-3a+ CTL2 and HNA-3b+ CTL2 in future. Further cell lines that express other HNA antigens

are also being developed which may replace granulocytes in immunofluorescence tests (GIFT).

#### Question 6

The UK Serious Hazards of Transfusion Scheme (SHOT) is a UK wide haemovigilance scheme in which TRALI has been included as a category since 1996. The overall total number of adverse events reported to SHOT has steadily increased each year but case reports of suspected TRALI have reduced since the introduction of preferential use of male plasma in late 2003 [1]. The most recent annual SHOT report shows a reduction from 36 reports of suspected TRALI in 2003 to 13 in 2010 [2]. The number of reported deaths, at least possibly due to TRALI, has also reduced from 27 in the 7-year period from 1997 to 2003 to 10 in the 7-year period from 2004 to 2010 [2].

A total of 164 cases reported to SHOT as suspected TRALI, over the last 14 years, have had complete antibody investigations. Of these, 101 (62%) have been found to have concordant donor HLA or granulocyte antibodies.

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## United States

Strategies for reducing the risk of TRALI from high plasma volume components were addressed in an AABB Association bulletin issued in November 2006 [1]. The bulletin stated that 'blood collecting facilities should implement interventions to minimize the preparation of high plasma volume components from donors known to be leucocyte alloimmunized or at increased risk of leucocyte alloimmunization'. The specific components designated as high plasma volume were whole blood, fresh frozen plasma (FFP), plasma frozen within 24 h after phlebotomy (FP24), plasma-cryoprecipitate-reduced, apheresis platelets and buffy-coat platelets resuspended in plasma. While the AABB recommended this general approach, the specific methods to achieve this were left to the discretion of individual institutions.

In order to determine the specific policies adopted by different blood collection agencies in the United States, AABB conducted a web-based survey in late 2009. These AABB survey results represent the most recent US national data [2]. Responses were received from 47 US blood collection agencies, including the three largest (American Red Cross, United Blood Services and New York Blood Center). Responding institutions collected 1.57 million apheresis platelet doses and 3.45 million plasma doses targeted for transfusion.

The American Red Cross, the single largest supplier of plasma and apheresis platelet components in the United States, had an ongoing haemovigilance programme in place to track the occurrence of TRALI reactions before and after the implementation of mitigation strategies and their findings are highlighted in this report.

### Question 1

The AABB survey revealed that plasma risk reduction policies were implemented by 46 of 47 responding blood centres. There was substantial variation between blood centres in selection of a gender-based plasma policy. There was also variation based on blood group (O/A vs. AB) and method of collection (whole-blood-derived or automated apheresis). For Group O/A plasma, most blood centres (51%) used male-only plasma, some (29%) used plasma from males and never-pregnant females, and others (18%) used predominantly male plasma. Although the survey did not specifically solicit information about whether centres asked male donors about a history of transfusion, it can be inferred from responses to a survey question about HLA testing of apheresis donors (see below) that few, if any, centres used a transfusion history as an additional selection criteria for determining acceptable plasma for transfusion.

The percentage of centres supplying male-only plasma declined to 39% for Group AB apheresis plasma and to 24% for Group AB whole-blood-derived plasma. Correspondingly, an increased percentage (34–41%) of blood centres supplied Group AB plasma that included plasma from never-pregnant females and 19% performed HLA antibody testing on some of their female plasma apheresis donors.

The American Red Cross, in particular, distributes approximately 1.6 million units of plasma and 850 000 apheresis platelets for transfusion each year, representing about 40% of the US supply. The majority (~75%) of the plasma is in the form of plasma frozen within 24 h (FP24) of collection, is derived from whole-blood collections and is not leucoreduced. The rest is plasma frozen within 8 h (FFP) and < 5% of the total is derived from apheresis collections. In late 2006, the American Red Cross implemented their male-predominant plasma strategy, migrating from its historical use of ~50% plasma from male donors, to achieve >95% plasma from male donors by November 2007, in accordance with AABB recommendations. In 2011, >99.5% of all type O, A and B plasma and about 67% of type AB are from male donors. The transfusion status of male donors is not taken into consideration, based on data showing that there is no statistical difference in the prevalence of HLA antibodies in transfused vs. non-transfused males [3].

### Question 2

As reported for plasma (see Question 1), it can be inferred that very few, if any, respondents to the AABB survey used a history of transfusion as an additional selection criteria for acceptable male platelet apheresis donors. The first platelet additive solution (Intersol; Fenwal Inc., Lake Zurich, IL, USA) was approved by the Food and Drug Administration in 2010, but has found limited national use in the United States to date. The American Red Cross successfully completed a pilot programme and is currently evaluating its implementation strategy. This solution replaces 70% of the plasma in an apheresis platelet product and is not viewed as a sufficient intervention to prevent TRALI.

Currently, the American Red Cross collects ~70% of apheresis platelets from male donors and is in the process of implementing a strategy to test all new female apheresis platelet donors for HLA antibodies.

### Question 3

In late 2009, 43% of responding US blood-collecting agencies tested some platelet apheresis donors for HLA antibody. All centres performing HLA antibody testing screened for both class I and class II antibodies. No centres performed HNA antibody testing.

Only two small blood centres (total collections of 14 000 platelet apheresis units) tested donors with a lifetime history of transfusion. There was wide variation among blood

centres with respect to the triage criteria (i.e. the number of pregnancies) used to determine whether a female donor would be tested for HLA antibody. Two blood centres screened all female donors, whereas 18 blood centres specified a certain number of pregnancies to initiate screening. The most common scenario was to screen women with one or more pregnancies, but it was almost as common to require four or more pregnancies. To complete the picture, there also were a few blood centres using  $\geq 2$  or  $\geq 3$  pregnancies as their triage criteria.

It is likely that data from the REDS-II Leukocyte Antibody Prevalence Study (LAPS-I) influenced the decision not to perform HLA antibody testing on male platelet apheresis donors with a history of transfusion. In LAPS-I, transfused blood donors did not have a significantly higher prevalence of HLA antibodies than their non-transfused counterparts; that is, HLA antibodies were detectable at low prevalence (1.0–1.7%) in male donors regardless of the transfusion history ( $P = 0.16$ ). A similar prevalence (1.7%) was found in never-pregnant female donors [3, 4].

Approximately 95% of platelets distributed by the American Red Cross are from apheresis collections, and the majority of these (~70%) are from male donors. In 2010, the American Red Cross began to screen female donors for HLA class I and class II antibodies if they (1) had a history of one or more pregnancies and (2) were new to apheresis platelet donation. Women with detectable HLA antibodies are deferred from apheresis platelet and plasma donation, but may donate whole blood. In these cases, the plasma derived from the whole-blood collection is sent for further manufacture into plasma derivatives.

#### Question 4

A primary method of TRALI mitigation in use by most US blood centres is the deferral of donors implicated in TRALI cases, although the efficacy of this intervention has not been proven to reduce TRALI incidence. Pathogen inactivation systems are not approved for use in the United States, and pooled platelets represent a small minority (~10%) of platelet components distributed by blood centres.

#### Question 5

Among AABB survey participants, four different HLA testing methodologies were used in 11 testing laboratories. The two most common assays used were the Luminex bead assay with One Lambda reagents (One Lambda, Canoga Park, CA, USA) and an ELISA (Donor Screen- HLA Class I and Class II on the Quick Step platform; GTI Diagnostics, Waukesha, WI, USA). Luminex testing allows a laboratory to set its own cut-off values, and each of the four laboratories using this assay system had set different cut-off values.

The class I NBG cut-off ratios used by the three laboratories were 59.3 (based on a 5 standard deviation cut-off from the mean as published by REDS-II investigators) [3], 30 and 10, while the class II NBG cut-off ratios were 27.5, 15 and 10. The fourth laboratory used a cut-off based on median fluorescent intensity.

Nineteen of 20 blood centres reported they would no longer accept individuals who tested HLA-antibody-positive as apheresis donors but instead would redirect these individuals to whole-blood donation. Red cells from subsequent donations were acceptable for transfusion but high-volume plasma components (e.g. FFP FP24) were not.

The American Red Cross utilizes the Luminex bead assay with One Lambda reagents (One Lambda) for HLA class I and II antibody detection.

#### Question 6

National US data on TRALI incidence are not routinely collected and were not available for consideration at the time that TRALI mitigation measures were introduced. In contrast, the American Red Cross National Hemovigilance Program, established in 2005, has systematically evaluated adverse reactions after blood donation and transfusion reported to American Red Cross blood centres [5]. Two strengths of the blood centre-driven haemovigilance programme are the centralized review of all reported reactions suspected to be related to manufacturing or donor selection, and the ability to capture and analyse the detailed information about every reported event and each involved donor. An inherent limitation of any passive surveillance system is that it likely underestimates the incidence of TRALI, given the difficulty in distinguishing TRALI from the more common causes of acute lung injury, and the likelihood that hospitals do not report all transfusion reactions to the blood centre. Regardless, data from the American Red Cross provide information on the baseline risk of TRALI per distributed blood component and demonstrate the effectiveness of mitigation efforts.

In 2006, the American Red Cross investigated 264 reported cases of suspected TRALI and determined that 69 cases likely met the clinical definition of TRALI or possible TRALI [5, 6]. Among these cases, 55 involved the transfusion of a single type of component [32 cases (including 6 fatalities) with plasma components (FFP or FP24); three cases with apheresis platelets, 19 cases (including five fatalities) with RBCs; and one case with whole-blood-derived platelets]. Cases where multiple component types were investigated were analysed, but did not alter the estimates of the risk and are not considered further. Consequently, the rate of probable TRALI in 2006 associated with plasma, apheresis platelets or RBCs was ~ 20, 5 or 3 per million distributed components, respectively [5, 6]. As many as six reported fatalities each year were observed with plasma

from female, HLA- or HNA-antibody-positive donors, suggesting that considerable morbidity and mortality could be prevented with a focused mitigation effort [5].

In 2007, all regional blood centres in the American Red Cross began preferentially distributing plasma collected from male donors for transfusion to patients and diverting plasma collected from female donors for further pharmaceutical manufacturing into plasma derivatives. By November 2007, the entire system reached its goal of having 95% or more of transfusable plasma components (FP24, cryoprecipitate-reduced plasma and FFP) from male donors and has maintained this practice in each subsequent year to date. In 2008, the rate of probable TRALI associated with plasma components, apheresis platelets or RBCs was ~ 4, 10 and 2 per million distributed components, respectively [6]. Compared to 2006, this represents a significant decrease in the number of probable TRALI cases attributed to plasma components in 2008 (32 cases vs. seven cases; OR 0.21; 95% CI: 0.08–0.45). Rates for apheresis platelets and RBC were not significantly different over this time period; notably, there were six fatalities in 2006 and five fatalities in 2007 involving plasma from female donors, but none in 2008 (2006 vs. 2008,  $P = 0.0013$ ).

Determining the number of cases that definitely implicated a specific donor antibody (e.g. cognate donor antibody-recipient HLA match) was limited by incomplete investigation of the recipient's HLA type in many cases, although it is reasonable to putatively implicate donors with broadly reactive antibodies or high PRAs (> 75%). When the recipients' HLA type was known, a cognate match with donor antibody was confirmed in the vast majority (88%) of cases [6]. An ongoing challenge is meeting the demand of AB plasma with exclusively male donors or 'reduced risk' screened (i.e. never-pregnant) or tested (i.e. HLA-antibody-negative) plasma. Currently, the residual risk of TRALI after plasma transfusion is almost exclusively due to the transfusion of AB plasma from antibody-positive female donors (A. F. Eder & R. J. Benjamin, American Red Cross Hemovigilance Program, unpublished data).

In conclusion, the number of TRALI cases that involved plasma transfusion was significantly reduced by about 80% after implementing a male-predominant plasma strategy, although some challenges remain. Additional strategies to mitigate the risk of TRALI with apheresis platelets are underway at the American Red Cross, but their effectiveness will be more difficult to evaluate considering that only a small number of TRALI cases occur each year.

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## Question 1

There is no Food and Drug Administration (FDA) requirement or recommendation for the exclusive use of male donors for FFP or other large plasma-volume products. However, regulatory intervention has not been thought urgent in consideration of AABB's release of a bulletin in 2006 advising blood establishments to minimize the preparation of plasma from donors known to be leucocyte-alloimmunized or at increased risk of leucocyte alloimmunization [1]. FDA has discussed TRALI and related mitigation measures at Blood Products Advisory Committee (BPAC) meetings. FDA convened a BPAC meeting in 2001 upon noting an increase in the incidence of TRALI-related deaths [2]. BPAC was asked at that time whether FDA should consider regulatory interventions to identify donors or donations with an increased risk for causing TRALI in a recipient. A majority of the BPAC members (13-1) voted 'no' to this question based on scientific uncertainties at the time. However, they recommended that further research be

conducted investigating the pathogenesis of TRALI and identifying best methods to mitigate this transfusion complication. In response to the BPAC recommendation, FDA published a health alert as a 'Dear Colleague' letter to the blood community in October 2001 in order to raise awareness of the diagnosis and treatment of TRALI as well as to stimulate the adverse event reporting [3]. In April 2007, BPAC again discussed current data on TRALI and agreed unanimously that the use of predominantly male plasma would reduce the incidence of TRALI [4]. A FDA policy on exclusive use of male donors for FFP and other large plasma volume products (e.g. apheresis platelets), while under consideration, has raised concerns about feasibility.

#### Question 2

On 9 December 2009, FDA approved the use of InterSol (Intersol, Fenwal Inc., Lake Zurich, IL, USA) as a storage solution for Amicus™ (Fenwal Inc., Lake Zurich, IL, USA) derived leucocyte-reduced platelets [5]. This is the only platelet additive solution currently approved in the United States. Intersol is an isotonic solution designed to replace a proportion of the plasma used in the storage apheresis platelets under standard blood banking conditions. Using this storage solution, the apheresis platelets are stored in a mix of 65% Intersol and 35% plasma. There is no FDA requirement or recommendation for the preferred use of male donors for apheresis platelets.

#### Question 3

FDA has cleared kits for HNA and HLA antibody testing. However, there are no FDA guidances or regulations regarding screening blood donors for HLA or HNA antibodies.

#### Question 4

FDA has not required or recommended measures that might prevent TRALI, nor have we approved any applications for pathogen inactivation of blood products. However, the agency encourages continued research in this area and welcomes submissions from interested manufacturers. We have cleared test kits for HNA and HLA antibody testing, and we do not object to preferential selection of male donors to prepare FFP and other large plasma volume products.

FDA is involved in collecting surveillance information on transfusion-related fatalities and monitors reports of deaths attributed to TRALI. FDA regulations require the reporting of fatalities related to blood transfusion, including TRALI (21 CFR 606.170 (b)). In this regulation, FDA requires transfusing facilities to notify the agency as soon as possible when a complication of blood transfusion is confirmed to be fatal. A team of FDA medical officers reviews these reports and determines whether there is a relationship between the blood transfusion and the reported fatality. These data allow FDA and blood establishments to monitor the effects of

TRALI mitigation measures. The summary reports for the years 2005 to 2010 are available on the FDA website [6].

FDA published a health alert as a 'Dear Colleague' letter to the blood community in October 2001. The letter reminds physicians to include TRALI in the differential diagnosis of a patient experiencing respiratory distress during or following a transfusion [3]. It also recommends prestorage leucocyte reduction of blood products to help prevent the formation of leucocyte antibodies in recipients. FDA also encourages voluntary Med Watch reporting of non-fatal TRALI cases and has published articles to raise clinical awareness of TRALI.

#### Question 5

FDA has cleared several HLA antibody assays that are based on enzyme-linked immunosorbent assay (ELISA) and flow cytometry platforms. FDA has also cleared HLA antibody assays performed on solid-phase, multiplex platforms such as the Luminex (Luminex Corporation, Austin, TX, USA). There is one FDA-cleared device that is Luminex-based for the detection of both HLA and HNA antibodies.

#### Question 6

TRALI fatalities reported to FDA have trended downward since 2005. Nevertheless, TRALI remains the leading cause of transfusion-related fatalities, representing 47% of confirmed transfusion-related fatalities reported over the past 6 years [6]. The number of TRALI-related fatalities associated with plasma products decreased from 23 (66% of TRALI cases) in FY 2006 to 4 (22% of TRALI cases) in FY 2010. This may be related to efforts by blood establishments to minimize the preparation of plasma components from donors known to be leucocyte-alloimmunized or at increased risk of leucocyte alloimmunization. While these trends are encouraging, it is likely that TRALI-related fatalities are underreported.

FDA does not always receive complete information on testing for HLA or HNA antibodies in TRALI-related fatality cases [6]. In FY 2006, investigators were able to match donor antibodies with recipient cognate antigens in eight cases. In FY 2007, matches were identified in seven cases, implicating 11 donors (information on the gender of implicated donors is unavailable). In FY 2008, matches were identified in four cases, implicating four female donors. In FY 2009, matches were identified in six cases, implicating five female donors and one male. In FY 2010, matches were identified in eight cases (information on the gender of implicated donors is unavailable).

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- (1) Bonfils Blood Center, Denver, CO, a smaller independent blood centre, collects plasma from males (transfusion history not known) and 'never been pregnant' females; such plasma is termed 'TRALI-mitigated' plasma. Strong Memorial Hospital of the University of Rochester Medical Center receives all of its transfusable plasma from the American Red Cross, which has a TRALI mitigation strategy in place that relies mainly on male donors. Blood Systems, which serves over 500 hospitals in 18 states, collects plasma using the same criteria as Bonfils Blood Center. Transfusion history is not obtained based on data showing no significant difference in levels of HLA antibodies in transfused compared with non-transfused males [1].
- (2) No routine plasma reduction is performed on platelet concentrates, and no platelet additive solutions are used by the independent centres in Denver, CO or Rochester, NY. Blood Systems collects all platelets via apheresis, and prestorage leucoreduces the products. Platelet additive solutions are not yet in use at Blood Systems, although their evaluation is being considered. Previously transfused donors are not deferred from platelet donation.
- (3) Donated whole blood from previously pregnant females is not used to manufacture plasma in Denver, CO. Previously pregnant females and transfused males are not

provided by suppliers for Rochester NY, except for some AB plasma, and such donors are not routinely screened for HLA or HNA antibodies. Importantly, about 50% of women who develop antibodies to HLA antigens postparturition become antibody-negative over time presumably due to a lack of re-immunization, and recent data demonstrated that 30/40 multiparous female donors with high-titre antibodies (>10-fold of the never-transfused male cut-offs) to HLA class I antigens, class II antigens or both by Luminex™ bead assays (Life Technologies; Grand Island, NY, USA) employing flow cytometry and ELISA testing lost these antibodies within 18 months (K. J. Land and D. R. Ambruso, unpublished data). These data are incongruent to previous data that demonstrated that such antibodies multiparous female donors were durable up to a decade postparturition. More data with regard to the longevity of such antibodies and their clinical ramifications are required. At Blood Systems, female platelet donors are asked whether they have ever been pregnant, and those women are screened for HLA class I and class II antibodies. Subjects with positive responses are deferred from platelet donation but are encouraged to donate red-blood-cells.

- (4) Strong Memorial Hospital, Rochester, NY, has long been investigating the utility of washing and prestorage leucoreduction of cellular components to decrease the pro-inflammatory effects of stored cellular components in the cardiovascular surgery setting and transfusion-related immunomodulation for patients with acute leukaemia [2-4]. Younger adults and some children with acute leukaemia (aged < 51 for myeloid leukaemia; aged 18-50 for lymphoid leukaemia), all neonates and all neonates and infants undergoing cardiac surgery with bypass at Strong Memorial Hospital routinely receive washed red cells and platelets by protocol based on randomized trials demonstrating improved clinical outcomes in most of these patient groups. After transfusion of close to 100 000 washed cellular blood components over the last 15 years, the incidence of reported TRALI and TACO after platelet and red cell transfusions has been zero, suggesting that the removal of stored supernatant is one possible strategy to mitigate both TRALI and TACO [3]. The Rochester transfusion service uses almost exclusively whole-blood-derived platelet pools, which we hypothesize represent a lesser risk of TRALI and other volume-related immunologic adverse effects. The Burn Unit at University Hospital, University of Colorado Denver has long used PRBC units stored < 21 days or washed if stored longer to decrease acute lung injury. A prospective study of TRALI performed at UCSF demonstrated that the elimination of high-risk plasma only decreased



plasma-related TRALI by 68% and the recent finding that platelet contamination of FDA-licensed plasma may allow the accumulation of sCD40L which could lead to TRALI suggests that whole-blood leucoreduction prior to component manufacture may represent a TRALI mitigation strategy in component processing [5, 6].

- (5) Smaller centres such as Bonfils in Denver will begin screening blood donors for leucocyte antibodies employing the GTI ELISA-based assay system. However, preliminary data have demonstrated that results for GTI and the Luminex™ bead-based flow cytometry tests for antibodies to HLA class I and class II antigens are not satisfactorily concordant (e.g. have low positive per cent agreement) even if assay cut-offs are adjusted based on results using samples from males who have not been transfused rather than using manufacturers' suggested cut-offs (K. J. Land and D. R. Ambruso, Unpublished data). Blood Systems screens for HLA antibodies using a Luminex-based technology, and samples that are positive at a level five standard deviations greater than those found in non-transfused males are deemed positive, which also reinforces that the cut-offs require adjustment [1]. Due to economic and throughput considerations, the GTI ELISA-based assays are being evaluated for implementation at Blood Systems.
- (6) As mentioned previously, data from Rochester have demonstrated that prestorage leucoreduction has significantly reduced the incidence of TRALI and TACO [3]. Similar data from Blood Systems, specifically UCSF, have demonstrated that the removal of high-risk plasma, by converting to the use of male-only plasma for transfusion, reduced TRALI by 68%; however, 32% of the observed TRALI reactions in this prospective cohort occurred using male-only TRALI-mitigated plasma that was antibody-negative [6]. In several haemovigilance systems, many cases of TRALI are due to red cell and platelet transfusions. Our preliminary data suggest that removal of stored supernatant immediately prior to transfusion has promise to dramatically reduce the cardiopulmonary complications of cellular transfusions [5, 6].

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