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## Human neutrophil antibodies in a blood donor population: a lookback study

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### Abstract

**Background and Objectives**—Human neutrophil antibodies (HNA) have been associated with severe transfusion-related acute lung injury (TRALI). We identified HNA antibodies in a blood donor population and performed an observational lookback on patients who received products from these donors to determine whether TRALI was associated with these transfusions.

**Materials and Methods**—Human neutrophil antibodies were determined in 1171 blood donors (388 non-transfused males, 390 human leucocyte antigen (HLA) antibody–negative females and 393 HLA antibody–positive females) for IgG and IgM antibodies using a flow cytometric assay. Selected positive samples had a monoclonal antibody immobilization of granulocyte antigen (MAIGA) and neutrophil genotyping performed to confirm specificity. Lookback was performed on patients receiving blood from donors with positive samples by extracting recipient data from hospital medical records. An expert panel of three pulmonary critical care physicians reviewed the summarized data and assigned a diagnosis of TRALI, possible TRALI, cannot distinguish between TRALI and TACO, TACO and other.

**Results**—Eight donors had HNA antibodies of which five contributed to this look-back (3-HNA-specific antibodies, 2-HNA non-specific antibodies). Seventy-six blood products were transfused from these donors into individual patients. One patient developed TRALI that was associated with a donor with a non-specific HNA antibody as well as class-I and class-II HLA antibodies.

**Conclusion**—The incidence of TRALI in this lookback was low and combined with low frequency of HNA antibodies in the donor population suggests not screening donors for HNA antibodies at this time is acceptable.

### Keywords

blood donors; HNA antibodies; lookback; TRALI

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### Introduction

Transfusion-related acute lung injury (TRALI) remains the leading cause of death from blood transfusion in the United States [1]. Both human leucocyte antigen (HLA) and neutrophil-specific antibodies have been implicated as the cause of the majority of cases of TRALI [2–5]. Many cases reported in the literature have found HLA antibodies in donor plasma but have not tested for the cognate antigen in the patient leaving open to question the role of the HLA antibody in a specific case. For those cases that have identified a cognate antigen, few have performed lookbacks to determine whether that donor antibody had been involved in other TRALI cases [6–9].

A recently published comprehensive review of TRALI cases demonstrated a significantly higher odds ratio for developing TRALI for patients who received a transfusion from a donor who tested positive for the presence of leucocyte antibodies compared with donors who did not have leucocyte antibodies [5]. However, there have been only a few lookback studies that focused on donors with known HLA antibodies who had not been implicated in a TRALI case [10–13]. The largest of these studies was the Leukocyte Antibody Prevalence Study-II (LAPS-II), a recently published multi-centre retrospective cohort which compared the incidence of TRALI in recipients of which half were transfused with components collected from anti-HLA-positive donors (study arm) and half from anti-HLA-negative donors (control arm) and matched by gender, parity and blood centre [11]. There was a slight increase in TRALI incidence in recipients of HLA antibody-positive units compared with HLA antibody-negative units (odds ratio of 3.6), although the difference was not statistically significant.

Only a few studies have involved a lookback on recipients who received blood components from donors with known neutrophil antibodies [14–16]. The most interesting was that of Kopko *et al.* [15] who reviewed the charts of 50 patients who received blood products from a donor with a human neutrophil (HNA) antibody that resulted in a transfusion-related fatality. She found 15 cases of pulmonary reactions from mild to severe most of which were never reported to the transfusion service or to the regional collection centre.

As part of the Leucocyte Antibody Prevalence Study-I (LAPS-I), we reported on the frequency and specificity of HNA in 1171 blood donors [17]. We now are reporting an observational lookback study on patients who received 76 blood products from five LAPS-I blood donors with documented HNA antibodies to determine whether TRALI was associated with these transfusions.

## Materials and methods

This study is an extension of the LAPS-II study that performed lookback investigations on patients receiving blood from donors with HLA antibodies. [11] LAPS-II was conducted as part of the National Heart Lung and Blood Institutes (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II) programme. The REDS-II co-ordinating centre (Westat, Rockville, MD, USA) and five REDS-II blood centres participated in LAPS-II. These centres included the American Red Cross Southern Region (Douglasville, GA, USA), Blood Center of Wisconsin (Milwaukee, WI, USA), Blood Centers of the Pacific (San Francisco, CA, USA), Hox-worth Blood Center/University of Cincinnati Academic Health Center (Cincinnati, Ohio) and the Institute for Transfusion Medicine (Pittsburgh, PA, USA). Each blood centre recruited a number of hospitals in its region to participate in this study with a total of 42 hospitals overall participating.

The methods used to determine the frequency and specificity of the HNA antibodies in a subset of LAPS-I donors have been previously described [17]. Briefly, 1171 donors (388 non-transfused males, 390 HLA antibody–negative females and 393 HLA antibody–positive females) were tested for IgG and IgM HNA antibodies. Testing was performed using a flow cytometric assay with additional testing on selected samples by a monoclonal antibody immobilization of granulocyte antigen assay and neutrophil genotyping. Eight samples were HNA antibody positive.

Five of the eight donors had components available for lookback that were distributed to hospitals participating in LAPS-II. Traced components included those from the HNA antibody–positive donation, those donated within the 5 years prior to the index donation, and those donated subsequent to the index donation but prior to the end of the study. In contrast to the larger LAPS-II study that included only high plasma volume components (transfusable plasma and plateletpheresis), this study also included red cells, whole blood–derived platelets, cryoprecipitate and whole blood. Of the 81 HNA-positive components identified, 9 had previously been investigated as part of the larger LAPS-II study because these were high plasma volume components from two donors with both HLA and HNA antibodies (see below).

The methodology used for the clinical determination of TRALI in this recipient lookback study has been previously described in detail [11]. In brief, recipient data were extracted from hospital medical records and included medical, radiographic and transfusion service records. Only patients having a chest x-ray within 24 h following the implicated component transfusion were further evaluated. The verbatim chest x-ray report was then reviewed by the principal investigator at each site to determine whether the x-ray was compatible with pulmonary oedema. If yes, a more extensive medical chart review was completed to determine whether hypoxemia was present. Those charts with a clinical finding of hypoxemia were then referred to a single critical care physician. If that physician determined that there was evidence of new or worsening pulmonary oedema that might represent a diagnosis of TRALI, then a detailed sequential documentation of the events surrounding the transfusion was made. This detailed report, along with chest x-ray data, was then passed on to an expert panel of three pulmonary/critical care specialists. The expert panel assigned one

of the following diagnoses to the cases they reviewed: TRALI, possible TRALI, cannot distinguish between TRALI and TACO (designated as TRALI/TACO), TACO and Other.

## Human subjects

The LAPS-II study was approved by the IRB of each centre and by the Westat IRB.

## Results

Table 1 summarizes the product and laboratory testing data on each of the five donors involved in the lookback. Two donors contributing 48 evaluable components had HNA-1a antibodies, one donor with 10 evaluable components had an HNA-4a antibody, and two donors with 18 evaluable components had HNA antibody without any definable specificity, reacting with some but not all of the HNA panel cells. One donor (Table 1, donor 4) with a non-specific HNA antibody also had HLA class-I and class-II antibodies of multiple specificities, and another donor with anti HNA- 4a (Table 1, donor 3) had HLA class-II antibodies. Three additional HNA antibody–positive donors detected in LAPS-I did not contribute to this lookback study because two were first time donors and the third had donated at a REDS site that participated in LAPS-I but not in LAPS-II.

Of the 81 blood products involved in this lookback, 46 were plasma, 22 were red cells, 8 were whole blood–derived platelets, 4 were cryoprecipitate, and 1 was whole blood. Five components were not transfused, leaving 76 evaluable products. Figure 1 describes the results for LAPS-II component tracing and recipient medical record review. Forty-eight patients had a 24 h post-transfusion chest x-ray with 13 demonstrating either new or worsening pulmonary oedema. Only one of these patients was hypoxemic, and this patient developed TRALI after transfusion of a plasma unit. The donor involved in this case had a non-specific HNA antibody as well as both class-I and class-II HLA antibodies of multiple specificities and had been reported as a TRALI case in the major LAPS-II manuscript. The recipient in this case did not have HLA or HNA antigen typing performed. Nine other recipients received blood components (3 plasma and 6 RBC) from this same donor, and none developed TRALI. There were no possible TRALI cases, and no TACO cases identified.

## Discussion

In this observational study of recipients of blood components from donors with HNA antibody, only one case of TRALI was observed in 76 lookback investigations. However, it is unknown whether HNA antibody was causative in this case because the donor also had a large array of class-I and class-II HLA antibody specificities and the recipient was not typed for HLA or HNA antigens. We believe that the non-specific nature of the HNA antibody argues for TRALI having occurred either owing to the HLA antibody mechanism or through a non-antibody-mediated mechanism rather than through an HNA antibody–mediated mechanism. Transfusion of HNA antibodies, particularly HNA-3a, can result in serious morbidity and mortality. Devoran *et al.* reported two cases of fatal TRALI associated with HNA-3a antibodies [18]. Reil *et al.* [2] reported 10 cases of TRALI from donors with HNA-3a antibody, six of which were fatal. Neither study reported lookback data on their

donor's other donations. Kopko *et al.* [15] described a fatal case of TRALI in which the donor had an HNA-3a antibody. She performed a lookback on 50 patients who received blood components from this donor within 2 years from the transfusion fatality. Although no additional fatalities were reported, there were 15 occurrences of mild to severe TRALI with two of the eight most severe reactions not being reported to the transfusion service. Only two of the 15 cases were reported to the regional blood collection centre. Muniz *et al.* [16] reported a case of TRALI following a platelet transfusion and demonstrated an HNA-3a antibody in the donor plasma. They noted that 11 other platelet components from this donor were transfused into patients prior to the TRALI event. Nine recipients experienced no adverse event, while the patient who had the initial TRALI reaction received two other platelet transfusions from this same donor. In one transfusion, the patient developed chills, while with the other transfusion the patient again developed evidence of TRALI. Fadeyi *et al.* [14] described two patients each of whom developed a combination of chills, rigours and dyspnea as well as transient leukopenia following transfusion from an HNA-2a antibody-positive apheresis platelet component that was divided into two separate units. The donor had donated 27 apheresis products resulting in 39 separate transfusions. A lookback on the recipients of these transfusions demonstrated 12 transfusion reactions in nine patients. All were mild to moderate in nature, and all occurred within 2.5 h of the transfusion with nine of the reactions resulting in pulmonary symptoms. In addition to these reactions, leukopenia occurred in 18 of 36 evaluable patients. These three reports emphasize that patient factors probably make a significant contribution to whether a neutrophil antibody results in a transfusion reaction. They also document that pulmonary reactions following blood transfusions frequently go unreported and that mild to moderate clinical reactions uncommonly result in the evaluation of the donor for leucocyte antibodies.

The role that non-specific neutrophil or HNA-1a antibodies play in TRALI is not well understood. During the period of 1996–2006, the UK SHOT system reported seven cases of TRALI associated with non-specific neutrophil antibodies and five cases associated with HNA-1a antibodies [3], and Reil *et al.* [2] reported one case of TRALI associated with HNA-1a. However, neither of these reports established causation. In our study that evaluated 48 recipients of components from HNA-1a antibody-positive donors and 18 recipients of components from donors with non-specific HNA antibody, we detected only one case of TRALI, which occurred in a recipient transfused with a non-specific HNA antibody and coexisting HLA antibodies. Based on the frequency of the HNA-1a antigen in the general population and the significant degree of reactivity of our non-specific antibodies against cells with various HNA antigens, it is clear that cognate antigens would have been present on the neutrophils of most patients transfused with these components in this study. Thus, if these antibodies do cause TRALI in patients with cognate antigens, they do so infrequently. Finally, we did not detect TRALI in 10 recipients of components from an HNA-4a-positive donor, and we are unaware of any such cases in the literature.

This study is the largest HNA antibody lookback study reported to date. Unlike several other studies, it evaluated recipients from multiple donors rather than from a single HNA antibody-positive donor. Nevertheless, the study has several limitations. Because the recipient record review protocol was designed to detect TRALI, patients without significant

post-transfusion changes in chest x-ray were not further reviewed. Thus, unlike other HNA antibody lookback studies, patients who experienced mild or moderate pulmonary reactions most likely would have been missed. However, the incidence of abnormal post-transfusion chest x-rays in our study (13 of 48: 27%) was similar to that of a group of controls without antibody (117 of 603: 20%) [11]. Second, we did not identify any donors with HNA-3a antibody, the specificity associated with many HNA antibody-mediated TRALI reactions and especially those with the most severe outcomes.

Most collection centres in the United States are producing plasma components from male only donors or from females who have never been pregnant with apheresis platelet products coming from males or females screened for HLA antibody. The plasma strategy has resulted in a decrease in the incidence of TRALI [3, 19–21]. As far as we know, no centres are testing their donors for neutrophil-specific antibodies in the United States. The low incidence of TRALI in this lookback study combined with the low frequency of HNA antibodies in the donor population suggests that this strategy of not testing for neutrophil antibodies at the present time is acceptable. However, further studies on the role of HNA antibodies in TRALI reactions need to be performed, particularly as these relate to HNA-3a antibody.

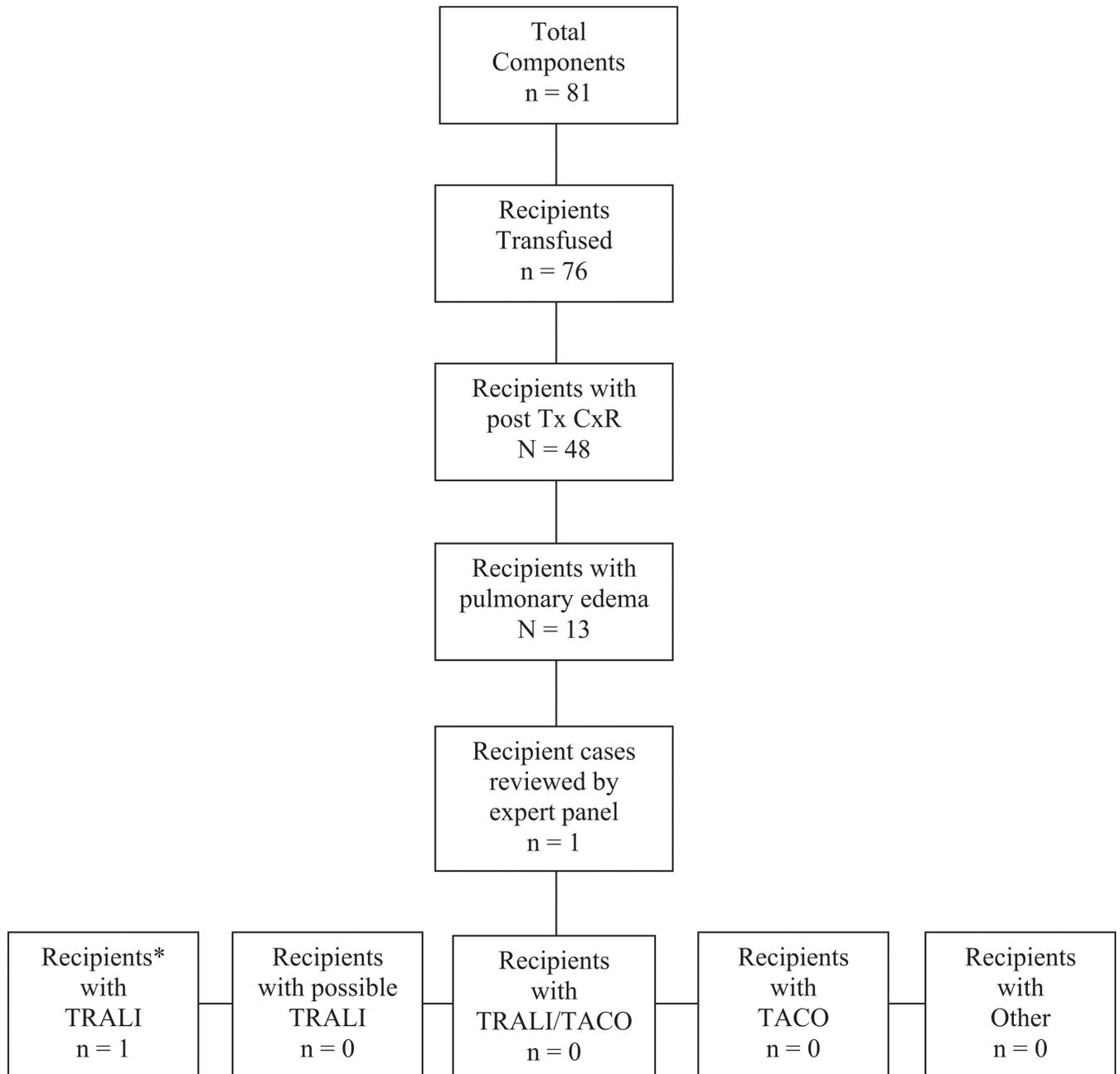
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**Fig. 1.** Components for HNA lookback: medical record review. \*Received product from donor 4, Table 1.

Table 1

Donor product and laboratory data

Donor	HNA antibody specificity	HLA CL I antibody	HLA CL II antibody	Number of lookbacks <sup>a</sup>	Number of units not transfused <sup>b</sup>
1	Ia	No	No	28	4
2	Non-specific	No	No	9	1
3	4a	No	Yes <sup>b</sup>	10	0
4	Non-specific	Yes <sup>c</sup>	Yes <sup>c</sup>	10	0
5	Ia	No	No	24	0

HLA, human leucocyte antigen; HNA, human neutrophil antibodies.

<sup>a</sup>There were 81 blood components investigated of which 76 were transfused. Component types were 46 plasma, 22 RBC, 8 platelets, 4 cryoprecipitate, 1 whole blood as follows: Donor 1: 28 plasma, Donor 2: 2 plasma, 7 RBC; Donor 3: 4 plasma, 4 RBC, 1 whole blood, 1 cryoprecipitate; Donor 4: 4 plasma, 6 RBC; Donor 5: 8 plasma, 5 RBC, 8 platelets (from whole blood), 3 cryoprecipitate.

<sup>b</sup>Donor 3 antibody specificities: no specific antibody identified.

<sup>c</sup>Donor 4 antibody specificities: Class I: B13, B15, B27, B40, B41, B44, B45, B46, B47, B49, B50, B53, Cw1, Cw3, Cw4, Cw14, Cw18; Class II: DR3, DR8, DR11, DR12, DR13, DR14, DQ6, DQ8, DQ9.