Immune mediated TRALI

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Transfusion Related Acute Lung Injury (TRALI)

Death by TRALI

Transfusion-related acute lung injury (TRALI), first reported in the early 1990’s, is a life-threatening immune reaction following a blood transfusion. It is now known that TRALI causes hundreds of deaths each year. Experts, however, suspect that the numbers are much higher, as many health-care workers do not recognize the symptoms. Although it is not clear what causes the reaction, according to the magazine New Scientist, the blood that causes it “appears to come primarily from people who have been exposed to a variety of blood groups in the past, such as . . . people who have had multiple transfusions.” One report states that TRALI is now near the top of the list for causes of transfusion-related deaths in the United States and Britain, making it “a bigger problem for blood banks than high-profile diseases like HIV.”
What is Transfusion-related Acute Lung Injury?

Acute respiratory distress syndrome (ARDS) occurring during or within six hours after blood products administration

Acute Lung Injury

- Hypoxemia
- New bilateral chest X-ray infiltrates
- No evidence of volume overload

With estimated rate of fatalities is 5 – 10%

Transfusion Related Fatalities
Reported to FDA 2014

<table>
<thead>
<tr>
<th>Cause</th>
<th>% of fatalities</th>
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<tbody>
<tr>
<td>TRALI</td>
<td>41%</td>
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<tr>
<td>Microbial infection</td>
<td>8%</td>
</tr>
<tr>
<td>HTR</td>
<td>21%</td>
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<tr>
<td>TACO</td>
<td>22%</td>
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![Bar chart showing the number of fatalities by cause and fiscal year (FY) from FY10 to FY14.]

- **TRALI**: 18, 10, 17, 14, 13
- **HTR (non-ABO)**: 5, 6, 5, 5, 4
- **HTR (ABO)**: 2, 3, 3, 1, 4
- **Microbial Infection**: 2, 4, 3, 5, 1
- **TACO**: 8, 4, 8, 13, 5
- **Anaphylaxis**: 4, 2, 2, 0, 5
- **Other**: 1, 1, 0, 0, 2

The chart shows the number of fatalities by cause and fiscal year from FY10 to FY14.
Non-immune vs. immune TRALI

- Transfusion reactions caused by chemical and physical agents (15% of TRALI cases)
  - lysophosphatidylcholines
  - interleukins
- Immunologic blood transfusion reactions (85% of TRALI cases)
  - anti-HLA class I
  - anti-HLA class II
  - anti-HNAs (HNA-2 and HNA-3a)
### Frequency of HNA and HLA antibodies in TRALI cases

<table>
<thead>
<tr>
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<th>death cases</th>
</tr>
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<tr>
<td>HLA class I</td>
<td>4</td>
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<td>HLA class I+II</td>
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<tr>
<td>HLA class II</td>
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<td>HNA-1a</td>
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<td>0</td>
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<tr>
<td>HNA-2</td>
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<td>10</td>
<td>6</td>
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<tr>
<td>Total</td>
<td>36</td>
<td>10</td>
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Reil et al, Vox Sang 2008
From where antibodies enter blood products?

Mechanism of alloimmunization during pregnancy

Exposure of immune system of mother (antigen negative) to fetus neutrophils (antigens Potive) induces production of allo(iso)antibodies against fetus. In neonatal alloimmune neutropenie, maternal immunoglobulin (Ig)G antibodies against a paternally inherited HNA cross the placenta and destroy fetal neutrophils (NIN). Transfusion of maternal plasma to antigen positive patients can leads to TRALI.
What evidence is there for non-immune TRALI

- Rats pretreated with LPS got ALI with lipids extracted from stored 42 day old human RBCs or platelets
- Did NOT get ALI if given lipids from 5 day product
- Thus plasma or lipids from fresh, human RBCs or platelets did not cause lung injury.
Immune TRALI: Mechanisms

Antibodies in blood components

HLA Class II

HLA & HNA

Gran

Cell priming by underlying diseases

HLA Class I

HNA?

Leak of protein rich fluid

Diapedesis

EC

Bux & Sachs, 2008
Acute lung injury mechanisms after antibody transfusion

Antibody theory animal models (1)

- Isolated perfused rat lungs perfused them with plasma containing anti HNA-2a mAb and human neutrophils.
- If neutrophil expression of HNA-2a antigen was >70%, ALI was manifest;
- If antigen expression was <30%, ALI was not manifest.
- However, if the lungs were primed with fMLP then lung injury was induced in the group with < 30% antigen expression.
- Also demonstrated that anti-HNA 2a directly activates neutrophils in the absence of complement

Anti-neutrophil Antibodies (HNA-2a)

HNA-2a > 70%

HNA-2a < 30%

More expression of HNA-2a on neutrophils enhances TRALI development after treatment with anti-HNA-2a

In ex vivo isolated rabbit lung model severe lung vascular leakage was reproduced using a human anti-HNA-3a antibody in the presence of human 3a-positive neutrophils.

In contrast, no vascular leakage was noted in lungs perfused in the absence of either antibody, neutrophils, or complement source.

No permeability increase occurred with the use of 3a-negative neutrophils.

Evidence for antibody theory (1)
Evidence for antibody theory (2)

- An in vivo mouse model BALB/c wild-type mice positive for MHC Class I antigen were injected with MHC Class I mAb and sacrificed at 2 hours. Control mice were knock out mice negative for the antigen.
- Injection of MHC Class I mAb produced severe ALI in wild type mice with 50% mortality compared to controls negative for the cognate antigen.
- The MHC Class I mAb binds throughout the body and prominently in the lung microvasculature.
- The investigators demonstrated that this antibody also binds to neutrophils, but does not cause direct neutrophil activation.

Evidence for antibody theory (2)

- Neutrophil depletion in mice resulted no TRALI!
- Fc-ko mice developed no TRALI!
- Thus in this model
  - MHC Class I antibody binds to class I antigen on the endothelium
  - Then presents the Fc portion of the antibody to the neutrophil
  - Neutrophil is then activated through Fc gamma receptor engagement.

Evidence for antibody theory (3)

- Incubation of serum containing anti-HLA class II antibodies with antigen positive monocytes increases the percentage of cytokine-positive cells.
- Anti-HLA class II induces release of leukotriene B4 (LTB4) from antigen-positive monocytes.
- Perfusion of isolated rat lungs with supernatant of activated monocytes and human neutrophils induces lung edema.
- Binding of HLA class II antibodies to monocytes induces release of neutrophils activating substances and therefore damage endothelial cells.

Kopko et al, Transfusion 2003
Sachs et al, Blood 2011,
Bayat & Sachs, Curr Pharm Des 2012
Two event hypothesis

1st event: Underlying clinical condition of patient (inflammation, infection, surgery)

Activation of pulmonary endothelium with increased adhesion molecules

2nd event: Transfusion of biologically active lipids or antibodies

Activation

Release of substances causing pulmonary endothelial damage and capillary leak

Severe TRALI
Mild TRALI

Healthy individuals
Patients at risk

(a)
(b)

Strength of transfusion-related mediators

Activated Neutrophils/endothelial cells
Primed
Resting

Individual predisposition

Silliman et al. Blood 2003
Kleinman et al. Transfusion 2004
Sachs, Current Opinion in Hematology 2011
Summary so far

Neutrophils play essential role for antiboides mediated TRALI

Pre-condition of endothelial cells play important role for development of antibody mediated TRALI (two-hit model)

Is there any evidence for direct role of endothelial cells in TRALI?

- Right, single lung transplant
- 10 weeks after transplant, transfused PRBCs for anemia
- HLA B44 antibody in PRBC donor
- HLA B44 antigen present in lung donor tissue only
- Elegant clinical example of targeted endothelial injury from a blood transfusion

## Frequency of HNA and HLA antibodies in TRALI cases

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Reil et al, Vox Sang 2008
Mechanism of HNA-3a antibodies mediated TRALI?

Not direct neutrophil activation!!

GAT: Granulocytes agglutination test, ROS: Reactive oxygen species, CTL-2: Choline transporter like protein 2
Human anti-HNA-3a alloantibodies react with mouse endothelial cells.

The first possibility to analyze the impact of human neutrophil antibodies for TRALI reaction in vivo.

Bayat et al. ATVB 2012.
**Human anti-HNA-3a antibodies induce ALI in vivo?**

- **Wild-type**
  - Human anti-HNA-3a → TRALI +
  - F(ab')$_2$ anti-HNA-3a → TRALI +

- **Neutrophils depleted**
  - Human anti-HNA-3a → TRALI +

- **Nox2$^{-/-}$**
  - Human anti-HNA-3a → TRALI -

**Direct binding of anti-HNA-3a to antigen positive endothelial cells Induces endothelial damages in a ROS-dependent pathway**

Bayat et al. ATVB 2012.
HNA-3 interact with Cochlin

Abundant inner ear protein
Multiple isoforms
Extracellular matrix component
Contain von Willebrand factor A domains

FCH: Factor C homology domain;
DNF: Deafness, autosomal dominant nonsyndromic sensorineura 9

Red : anti-CTL-2 (anti-HNA-3)
Green : anti-Cochlin

Kommaredi et al. 2007
Neutrophil aggregation induced by HNA-3a antibodies depends on plasma.

Protein 1.5pM; Antibodies 10nM; 37°C, 2h
GAT

Anti-vWF mediated GAT inhibition

Anti-vWF mediated inhibition of cell adhesion

Neutrophil ROS production

Bayat et al. ATVB 2015, Bayat et al, Transfusion 2012
Anti-HNA-3a mediated vWF dependent neutrophil aggregation

Relevance of GAT in anti-HNA-3a mediated TRALI??
Anti-HNA-3a induced TRALI in VWF deficient mice

Wild-type

VWF-KO

TRALI

TRALI
Possible mechanism of severe TRALI induced by anti-HNA-3a

1) Anti-HNA-3a
2) ROS
3) Permeability disturbance
4) More permeability disturbance
5) vWF
6) vWF
7) ROS, NET, Proteases
8) vWF
9) endothel

Bayat et al. ATVB 2013.
Mechanism of antibodies mediated TRALI

1. Step

1) Crucial
2) Antibodies
3) ROS
4) Proteins
5) Neutrophils

2. Step

1) CTL-2
2) VWF
3) ROS
4) Not Crucial
Summary

- Binding of anti-HNA-3a to CTL-2 on endothelial cells leads to endothelial dysfunction, this pathway represents the major cause of TRALI mediated by anti-HNA-3a antibodies.

- VWF mediates anti-HNA-3a induced neutrophil aggregation in vitro.

- In this process, CTL2 interacts with VWF via A1 domain leading to neutrophil aggregation.

- This reaction pathway, however, does not seem to play a significant role in vivo. Treatment of neutrophils depleted mice and VWF-KO mice with anti-HNA-3a antibodies led to TRALI.

- Stabilization of endothelial cells as treatment strategy for TRALI should be considered.
Transfusion-related acute lung injury in multiple traumatized patients

Abstract

Background: Many of the multiple traumatized patients who refer to the hospital need transfusion. Transfusion-related acute lung injury (TRALI) is a serious clinical syndrome associated with the transfusion of plasma-containing blood components. In the article, we present a case of TRALI following transfusion of packed red blood cells.

Case Presentation: A 24 year old male referred to Shahid Beheshti Hospital due to multiple trauma with left femoral and humerus fractures. Due to severe anemia he received 3 units of packed red blood cells. The symptoms of TRALI began 2 hours after transfusion. He was transferred severe hypoxia. The TRALI was 3 diseases. He recovered and was

Conclusion: Transfusion related receiving transfusion of plasma

Keywords: Transfusion, Lung

Many of the patients:

Evaluating medical interns’ knowledge of common blood transfusion complications

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<th>Number of respondents (%)</th>
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<th>Percentile</th>
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<td>Clinical symptoms of allergic reactions</td>
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<td>Management of allergic reactions</td>
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<td>Symptoms of TRALI</td>
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<td>Etiology of TRALI</td>
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<td>84 (93.3%)</td>
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<td>Preventing volume overload in heart failure</td>
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<td>84 (93.3%)</td>
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<td>15</td>
<td>Sum</td>
<td>20</td>
<td>90</td>
<td>10.94</td>
<td>51</td>
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TRALI: transfusion-related acute lung injury.
How to prevent TRALI

Since antibody are involved in majority of TRALI cases the following strategies must be considered:

- Establishment of HNA typing (SSP: Sequence Specific PCR)

- Identification of HNAs distribution in Iranian populations

- Establishment of antibody detection test (MAIGA: Monoclonal antibody immobilization granulocytes antigen)

- Antibody screening among female donors
# Acknowledgments

<table>
<thead>
<tr>
<th>Institute for Clinical Immunology and Transfusion Medicine, JLU, Giessen, Germany</th>
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<tbody>
<tr>
<td>Yudy Tjahjono</td>
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<td>Heike Berghöfer</td>
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<td>Ulrich Sachs</td>
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