Let’s Talk About Leukoreduction (Again)

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Objectives

• Currently accepted indications for leukoreduction
• Infrequently considered effects of donor white blood cells
• Future prospects

Current Indications: Prevention of HLA Alloimmunization and Platelet Refractoriness

• 1950’s: technological advancements allowed for preparation of platelet concentrates
• Shortly afterwards platelet refractoriness was recognized as a relatively common complication of chronic platelet transfusions

Platelet Refractoriness: Immune + Non-immune causes

70% = non-immune causes
<20%= immune causes

- HLA Class I Ab (80-90%)
- HPA (10-20%)
- Both (5%)

DIC
Drugs

Direct and Indirect Allorecognition

Direct:
T-cell receptor of recipient CD4+ T cells directly interacts with MHC II molecules on donor APC

Leukoreduction of platelets removes this pathway

Indirect:
Default pathway for MHC Class I:
Donor platelet derived MHC class I Ag taken up by recipient APC, process and presented by MHC class II molecules on recipient APC

T cell help and alloAb production
Trial to Reduce Alloimmunization to Platelets  
N Engl J Med 1997; 337:1861-1870

- 15 percent control group  
- 10 percent ultraviolet B–irradiated pooled platelet concentrates from random donors (UBV-PC)  
- 7 percent filtered, pooled platelet concentrates from random donors (F-PC)  
- 8 percent filtered platelets obtained by apheresis from single random donors (F-AP).

There were no significant differences among any of the treated groups (P values ranged between 0.42 and 0.76). The P values for the comparisons between the control group and the treated groups were 0.17 for the UBV-PC group, 0.03 for the F-PC group, and 0.06 for the F-AP group.

Current Indication: Reduction of Febrile Non-Hemolytic Transfusion Reactions (FNHTR)

- FNHTR: Occurs during or within 4 hours of cessation of transfusion AND EITHER  
  - Fever (greater than or equal to 38°C/100.4°F oral and a change of at least 1°C/1.8°F) from pre-transfusion value  
  OR  
  - Chills/rigors are present

(FHSN Biovigilance Component  
Hemovigilance Module Surveillance Protocol v2.2  
www.cdc.gov/nhsn)

Pathophysiology of FNHTR

- Leukocytes  
- Cytokines released during storage from leukocytes  
- Platelet derived soluble CD154 (CD 40 ligand)

Leukocyte derived  
Platelet derived

FNHTR platelets > FNHTR RBC

<table>
<thead>
<tr>
<th>Component</th>
<th>Rate range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>0.06-2.2/100 units</td>
</tr>
<tr>
<td>RBC</td>
<td>0.04-0.44/100 units</td>
</tr>
</tbody>
</table>

- Platelet derived soluble CD40 ligand  
- Type of component modification


Nonleukoreduced (NonLR) vs  
- SDP P = .008  
- PostSLR P = .045  
- PostSLR vs NonLR

Nonleukoreduced NonLR platelets in FNHTRs tended to be older (4.5 d)

Conclusions:  
- FNHTRs: PostSLR-NonLR vs SDP  
- The timing of leukocyte removal during platelet storage influences amount of cytokines and impacts FNHTRs
Current Indications: Reduce the risk of CMV transmission

CMV: A member of the herpesvirus family
Large DNA genome
Causes persistent and latent infection
Causes cell enlargement

CMV and Transfusion

- CMV positive cellular components transfused to recipient with normal functioning immune system has minimal impact
- Often asymptomatic
- CMV seroprevalence: 40-90% general population
- Therefore donor deferral impractical

CMV Infection

- **Immunocompetent:**
  - Asymptomatic or mild mononucleosis-like symptoms
  - Prevalence: 40-90% adults
- **Immunocompromised:**
  - Fever
  - Leukopenia
  - Thrombocytopenia
  - Hepatitis
  - Enteritis
  - Pneumonia
  - Increased risk renal allograft rejection
  - Impaired cellular immune response

CMV can be life threatening

- Very low birth weight neonates
- Fetus requiring intrauterine transfusion
- Pregnant women
- Primary immune deficiency
- Transplant recipients
- Chemotherapy or HPC transplant

Leukoreduced vs Seronegative

- With high prevalence in donors, finding CMV seronegative blood is very difficult
- Larsson et al (Transfusion, 1998): 55% CMV seronegative donors had CMV DNA detected in monocytes
- Serologic testing for CMV:
  - Relatively poor sensitivity
  - Serologic testing not useful in selecting blood that eliminates CMV infection
  - Transfusion transmission vs community acquired

- Why not Nucleic Acid Test for CMV?
  - CMV DNA primarily localized to monocytes and not plasma

Bedside Leukoreduction prevents TT-CMV

Bowden et al

Table 3. Incidence (and Actuarial Probability) of CMV Infection and Disease by Study Arm

<table>
<thead>
<tr>
<th></th>
<th>Seronegative Blood (N = 252)</th>
<th>Filtered Blood (N = 250)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CMV infections + disease</td>
<td>2 (1.3%)</td>
<td>3 (2.4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>CMV disease only</td>
<td>0 (0%)</td>
<td>3 (1.2%)</td>
<td></td>
</tr>
<tr>
<td>All CMV infections + disease</td>
<td>4 (1.6%)</td>
<td>6 (2.4%)</td>
<td>0.5</td>
</tr>
<tr>
<td>CMV disease only</td>
<td>0 (0%)</td>
<td>6 (2.4%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Survival 79% vs 82% 0.56

CMV infection was defined as the identification by culture or CMV antigen detection of CMV from any clinical specimen, and
CMV disease was defined as biopsy evidence of CMV in tissue with compatible clinical symptoms.
Nichols et al
TT CMV infection after receipt of LKR blood products
Blood 2003;101:4195-4200

- 807 CMV negative SCT patients
- Weekly CMV surveillance (pp65 antigenemia assay)
- Period 1: CMV seronegative and/or LKR
- Period 2: only LKR
- Higher incidence of TT-CMV during Period 2 (4% vs 1.7%, p<0.05), greater risk with RBC
- Pre-emptive Rx with ganciclovir prevented all but one case of CMV disease

Leukoreduction reduces the load of potentially infectious virus but does not completely eliminate CMV transmission

AABB Committee Report: reducing transfusion-transmitted cytomegalovirus infections

- Doi:10.1111/trf.13503
- Tried to develop clinical practice guidelines

They couldn’t ...

- Due to low quality of recent studies

International Survey TT-CMV prevention practices

<table>
<thead>
<tr>
<th>Country</th>
<th>Univ LR</th>
<th>CMV Ab Testing</th>
<th>NICU</th>
<th>IUT</th>
<th>Prog</th>
<th>SCT</th>
<th>Solid organ (tag)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>N</td>
<td>Y</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Australia</td>
<td>Y</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brazil</td>
<td>Y</td>
<td>Y</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Canada</td>
<td>Y</td>
<td>Y</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Switzerland</td>
<td>N</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>France</td>
<td>Y</td>
<td>Y</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3 (lung)</td>
</tr>
<tr>
<td>Germany</td>
<td>Y</td>
<td>Y</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>UK</td>
<td>Y</td>
<td>Y</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Leukoreduction decreases incidence of RBC alloimmunization

Transfusion 43 (7), 945-952

- RBC alloimmunization
- Transfusion related immunomodulation (TRIM)
- Other infectious diseases
  - HTLV
  - Prions

Other Impacts of Donor WBC

- RBC alloimmunization
- Transfusion related immunomodulation (TRIM)
- Other infectious diseases
  - HTLV
  - Prions

*An decrease in alloimmunization was observed in both males and females, with the decrease more evident in males.*
Transforming growth factor-β released by apoptotic white blood cells during red blood cell storage promotes transfusion-induced alloimmunomodulation. Vaillon et al, Transfusion 2015;55:1721-1735

Potential Mechanisms of Transfusion Related Immunomodulation (TRIM)

- Decreased helper T-cell count
- Decreased helper/suppressor T-lymph ratio
- Decreased lymphocyte response to mitogens
- Decreased Natural Killer (NK) cell function
- Reduction in Delayed-type hypersensitivity

- Defective antigen presentation
- Suppression of lymphocyte blastogenesis
- Decreased cytokine (IL-2, IF-γ) production
- Decreased monocyte/macrophage phagocytic function
- Increased production of antiidiotypic and anticonnotypic antibodies

Brunson ME, Alexander JW. Transfusion 1990;30:651-658

Alloimmunization

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Transfusion Effect

- Donor specific blood transfusion

http://www.diabetesmonitor.com/images/transplant.jpg

Graft Survival versus Transfusion Dose, 1978-82 UCLA Registry

P<0.001 all curves
n=1436
n=2652
n=2391

Leukocyte reduction diminishes this effect

HTLV Infection

- **HTLV1**
  - mainly affects CD4+ lymphocytes
  - adult T-cell leukemia (2-3%)
  - HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP, 1-2.5%)
  - Most infected remain asymptomatic carriers
  - Neuropathic disease, uveitis, Sjogren’s, polymyositis, aphthous stomatitis, nongenital warts

- **HTLV2**
  - affects CD8+ lymphocytes
  - HAM/TSP and increased neurologic disability
  - May impact platelet count and be responsible for pneumonia, bronchitis, arthritis, and dermatitis

HTLV and Leukoreduction

- **Virus integrated in lymphocytes, little cell free**
- **Number of infected wbc is critical to efficiency of transmission**
- **Laperche et al: 10 fold reduction in transmission risk with leukoreduction**
- **Sobata et al: quantitative PCR on 300 donations from HTLV-1 positive donors**
  - Analyzed distribution of proviral load
  - Estimated transmission cutoff of $9 \times 10^4$ HTLV-1 + wbc/product
- **Leukoreduced may be safer but risk remains in donors with high proviral load**
- **Cost effectiveness of HTLV screening**
  - Consider first time donors only (Sweden); France is considering
  - Finland and Norway: no longer test as of 2007/2008

Prion transmission and Leukoreduction

- The United Kingdom has shown the highest incidence of vCJD in the world: 177 definite of probable cases reported up until November 3, 2014
- 3 cases of vCJD in UK - transfused with nonleukoreduced red blood cells (RBCs) from donors who were later confirmed to have died from vCJD
- To reduce the likelihood of vCJD transmission via blood products, protective measures have been adopted in the United Kingdom, including donor deferral, importation of plasma, and leukoreduction

Odds ratios for post-op infection in surgical patients transfused Leukoreduced vs Non-leukoreduced

- **Less Infection: Favors Leukoreduced**
- **More Infection: Favors Non-Leukoreduced**

HTLV and HTLV-1 infection with transfusion safety. Transfusion Clinique et Biologique 2016;23:13-18

McCUTCHEON S et al. Transfusion 2015:55(9);2123-2133

- Non-LR RBC, 8/21 (38%) infection rate in recipients vs 3/12 (25%) LR RBC

"Of 14 recipients of LR-RBCs or LR-RBC-P-CAPT, we observed infective and clinical disease in two recipients of paired components from the same donor..." - The results indicate that, after leukoreduction and P-CAPT filtration, there can still be sufficient residual infectivity in sheep RBCs to transmit infection when transfused into a susceptible recipient."
Impact of Pathogen Reduction

- INTERCEPT Blood System (Cerus)
- FDA approved December 2014
- Photochemical treatment
- Amotosalen, UV-A light
- FDA approved:
  - Apheresis platelets (PAS-3)
  - Whole blood or apheresis plasma

INTERCEPT Package Insert

### Viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Log Reduction (pfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1, cell associated</td>
<td>5.4</td>
</tr>
<tr>
<td>DHBV (model virus for HBV)</td>
<td>≥4.8</td>
</tr>
<tr>
<td>BVDV (model virus for HCV)</td>
<td>≥4.4</td>
</tr>
<tr>
<td>HTLV-I</td>
<td>4.7</td>
</tr>
<tr>
<td>HTLV-II</td>
<td>≥5.1</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>≥5.7</td>
</tr>
<tr>
<td>Chikungunya virus (CHIKV)</td>
<td>≥5.7</td>
</tr>
<tr>
<td>Dengue virus</td>
<td>≥4.3</td>
</tr>
<tr>
<td>Cytomegalovirus (CMV)</td>
<td>≥4.9</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>≥5.9</td>
</tr>
<tr>
<td>Bluetongue virus</td>
<td>4.4</td>
</tr>
<tr>
<td>Adenovirus 5</td>
<td>≥4.9</td>
</tr>
</tbody>
</table>

### Protozoan Parasites

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Log Reduction (pfu/mL or cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmodium falciparum</td>
<td>≥5.6</td>
</tr>
<tr>
<td>Babesia microti</td>
<td>≥4.9</td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>≥5.3</td>
</tr>
</tbody>
</table>

Summary

- Reduction of risk of HLA alloimmunization and platelet refractoriness, prevention of FNHTRs, and reduction of risk of transmission of CMV are accepted indications for leukoreduction
- Leukoreduction also may be associated with
  - Reduced RBC alloimmunization
  - Reduced risk of HTLV and prion transmission
- Pathogen reduction may lead to a reduced need for leukoreduction however increased usage of PR is limited by economic factors

Questions?

Reduced alloimmunization in mice following repeated transfusion with pathogen-reduced platelets (Mirasol)

Balb/c mice transfused weekly for 2, 4, or 8 weeks with C57Bl6J PRP
A: Non leukoreduced PRP
B: Leukoreduced PRP

Antibody levels increased with number of transfusions, but were significantly reduced using either UV+R (riboflavin) or leukoreduction. Combining UV+R with leukoreduction was best protection.

*Platelet recovery was reduced with UV+R (not shown)