Medical Issues in a Clinical Cellular Therapy Laboratory

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Objectives

- Major, Minor and Bidirectional ABO Incompatibility in HSC transplants
- Blood components in the 3 stages of transplant
- Stem cell infusion reactions
- Engraftment failure
Hematopoietic Progenitor Cell Transplantation

Outcome of HPC transplant may be compromised with transfusion:

- Alloimmunization from HLA s with transfused products
- Immunohematologic consequences of ABO mismatched transplants
- Impact of immunosuppression associated with the HPC procedure
Hematopoietic Progenitor Cell Transplant

- Blood groups... a static characteristic for a given individual
- Stem cell transplantation... blood group may be dynamic; antigens expressed / antibodies present may change at different times
- Different cell lines engraft at different times and have different half-lives.. Chimera ( transient )
Transplantation Types

Donor source: autologous, syngeneic, allogeneic

- **Autologous transplants**: genetically identical
- **Syngeneic transplants**: healthy monozygotic twin; same HLA and ABO; minimal risk of GVHD
- **Allogeneic**: related or unrelated HLA matched / partial matched;
  ABO blood group pairings may be identical, major incompatible, minor incompatible, major and minor incompatible
Pre Transplantation Phase

When a patient is being considered for HPC transplant:

- Communicate HPC transplant candidate info to blood transfusion service: special blood components
- Gamma irradiation of cellular components
- Leukoreduced cellular components
- CMV safe/ CMV seronegative
- Family members should be avoided as blood donors to prevent alloimmunization against minor histocompatibility and private antigens increasing risk of graft rejection (future related transplantation less likely to be successful)
Provision of Blood Products

Autologous Transplant

- Indications as in patients with malignancy
- Leukoreduced products
- Gamma irradiated to prevent GVHD
- CMV seronegative patients receive CMV safe components
- Red blood cells preferably ABO identical or compatible
Provision of Blood Products

Allogeneic Transplant:

- When recipient is CMV seronegative, cellular blood products must be from CMV seronegative donors
- Irradiated and leukoreduced products
- Blood product support in **ABO identical** allo transplant: compatible blood products
- Special considerations in **ABO mismatched transplants**:  
  - Antigens expressed, antibodies present can differ at various stages of engraftment
Major and Minor ABO Incompatibility in HSC transplants
Allogeneic Donor Selection

- HLA matching is paramount
- HLA (chrom 6) and ABO (chrom 9) antigens are encoded by different genes
- HLA antigens are expressed on immature pluripotent stem cells, ABO antigens are not
- 30-50% of Allogeneic HPCT are ABO incompatible
**ABO-Incompatible HSCT**

- **Major:** recipient has ABO Ab directed against donor RBCs
  - A donor, O recipient

- **Minor:** donor has ABO Ab directed against recipient RBCs
  - O donor, A recipient

- **Bi-directional:** Ab going in both directions
  - A donor, B recipient
ABO incompatible stem cell transplants

- It is known that clinically significant hemolytic complications can occur at:
  - Infusion
  - Post transplant

- But, does ABO Incompatibility affect overall survival, non-relapse mortality or GVHD?
Incompatibility?

- Plasma
  (Recipient)

- RBCs
  (Graft)
Major Incompatibility

- Plasma (Recipient)
- RBCs (Graft)
ABO mismatched allo transplants: **Major incompatible transplant**

Introduction of foreign ABO antigen against which recipient has preformed antibody

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group O</td>
<td>Group A, B, AB</td>
</tr>
</tbody>
</table>

- Severe hemolysis during stem cell infusion, so RBC depletion during processing
- Residual recipient anti-A / anti-B continue to circulate with half life of 3 weeks
- Red cells be of recipient ABO group (? washed free of antidonor antibodies )
- Plasma components … donor ABO group
- After complete engraftment, red cells can be of donor type
Table 12-1. Transfusion Support Beginning at Myeloablation for Major ABO-Incompatible Hematopoietic Stem Cell Transplantation

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>(Washed) RBCs*</th>
<th>Platelets: First Choice</th>
<th>Platelets: Second Choice</th>
<th>Platelets: Third Choice</th>
<th>Platelets: Fourth Choice</th>
<th>Fresh Frozen Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>A</td>
<td>O</td>
<td>A</td>
<td>AB</td>
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<td>A, AB</td>
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<tr>
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<td>O</td>
<td>AB</td>
<td>A, B</td>
<td>0</td>
<td>-</td>
<td>AB</td>
</tr>
</tbody>
</table>

*Until the direct antiglobulin test is negative and antidonor iso-hemagglutinin is no longer detectable; thereafter, transfuse donor ABO group.
Problems with Major Incompatibility

- First stage: During infusion
  - **Problem**: Incompatible red cells infused with the HPC product may be hemolyzed by native ABO antibody in recipient
  - **Solution**:
    1) Red cell depletion of the product with goal of <20-30 mL RBCs in final product
    2) For very high titers in recipient, consider apheresis
Hemolytic Problems with Major Incompatibility

- Second stage: During Engraftment
  - **Problem:** Recipient ABO antibodies may persist and hemolyze newly formed incompatible red cells
  - **Solution:**
    1) Transfuse donor and recipient compatible red cells and plasma until reverse typing consistent with donor type
    2) For very high titers in recipient, consider apheresis
Engraftment Problems with Major Incompatibility

- Does NOT (generally) affect outcome of transplant
- Does NOT affect development or severity of GVHD
- Does affect red cell transfusion requirement
- Does affect time to red cell engraftment; white cells and platelets are (generally) unaffected
  
  Delayed red blood cell recovery / Pure red cell aplasia

Donor RBC chimerism was markedly delayed following reduced intensity versus myeloablative SCT (median, 114 versus 40 days; P < .0001) and strongly correlated with decreasing host anti-donor isohemagglutinin levels. (Bolan CD, Blood, 2001)
Incompatibility?

- Plasma (Donor)
- RBCs (Recipient)
Minor Incompatibility

- Plasma (Donor)

- RBCs (Recipient)
ABO mismatched allo transplants: Minor incompatible transplant

Introduction of foreign isohemagglutinin that targets red cell Ag of recipient

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Group O</td>
</tr>
<tr>
<td>Group B</td>
<td>Group O</td>
</tr>
<tr>
<td>Group AB</td>
<td>Group A, B, O</td>
</tr>
</tbody>
</table>

- Stem cell processing should include plasma depletion (washing); DAT may be positive
- Red cells must be of donor ABO group (*washed to remove antirecipient isohemagglutinin* until original rbc no longer detectable in the recipient)
- Plasma/ platelets… recipient type until donor cells engrafted
Table 12-2. Transfusion Support Beginning at Myeloablation for Minor ABO-Incompatible Hematopoietic Stem Cell Transplantation

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>(Washed) RBCs*</th>
<th>Platelets: First Choice†</th>
<th>Platelets: Second Choice</th>
<th>Platelets: Third Choice</th>
<th>Platelets: Fourth Choice</th>
<th>Fresh Frozen Plasma†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>O</td>
<td>O</td>
<td>A</td>
<td>AB</td>
<td>B, O</td>
<td>-</td>
<td>A, AB</td>
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<tr>
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<td>AB</td>
<td>B</td>
<td>A</td>
<td>0</td>
<td>AB</td>
</tr>
</tbody>
</table>

*Wash until recipient erythrocytes are no longer detectable.
†Until recipient erythrocytes are no longer detectable; thereafter, transfuse donor ABO group.
Problems with Minor ABO Incompatibility

- First stage: Infusion
  - **Problem**: Incompatible plasma infused with HPC product, may hemolyze recipient red cells
  - **Solution**: Plasma reduction decreases/prevents passive transfer of isoagglutinins (prevents acute hemolysis)
Problems with Minor ABO Incompatibility

- Second stage: Passenger Lymphocyte Syndrome
  - **Problem:** Viable B lymphocytes in HPC product produce isoagglutinins against residual recipient RBCs, 5-15 days post-transplant
  - **Solution:** Supportive RBC transfusion, RBC exchange (rare), Rituximab, Methotrexate
Passenger Lymphocyte Syndrome

- Detection:
  - Positive DAT
  - Donor derived isoagglutinins in serum and eluate
  - Only 10-15% of patients with + DAT develop hemolysis

- Hemolysis subsides as residual recipient RBCs are destroyed or replaced by donor or transfused RBCs
Does Minor ABO Incompatibility change the risk of GVHD?

- There may be higher risk of GVHD after minor ABO-incompatible transplant.
- A or B antigens expressed on endothelial and epithelial tissue of the host: target for GVH response.
ABO Mismatched

Combined Major & Minor

Incompatible

Allogeneic Transplants
**ABO mismatched allo transplants: Combined major and minor**

Introduction of both foreign antigens and isohemagglutinins

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>Group A</td>
</tr>
<tr>
<td>Group A</td>
<td>Group B</td>
</tr>
</tbody>
</table>

- Generation of novel antigen / antibody and loss of recipient antigen / antibody occurs at different stages
- Stem cell processing should include RBC depletion and plasma depletion to minimize hemolysis
- (Washed) group O RBC until DAT is negative
- AB plasma components until original recipient red cells not detectable
Table 12-3. Transfusion Support Beginning at Myeloablation for Combined Major and Minor ABO-Incompatible Hematopoietic Stem Cell Transplantation

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>(Washed) RBCs*</th>
<th>Platelets: First Choice†</th>
<th>Platelets: Second Choice‡</th>
<th>Platelets: Third Choice</th>
<th>Fresh Frozen Plasma†</th>
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<td>B, A</td>
<td>O</td>
<td>AB</td>
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</tbody>
</table>

*Until direct antiglobulin test is negative and antidonor iso-hemagglutinin is no longer detectable; thereafter, transfuse donor ABO group.

†Until recipient erythrocytes are no longer detectable; thereafter, transfuse donor ABO group.

‡Selection is dependent on red cells circulating in patient.
Laboratory Processing ABO I

- Red cell depletion (Goal < 20 - 30 ml red cells or < 0.4 mL/kg recipient weight)
- Plasma depletion (avoid acute hemolysis of recipient RBCs)
Role of Antibody Titers

- Anti-A or Anti-B
- Titer level depends on the individual’s immune system and prior exposure to ABO antigens
- Can be performed on donor (minor) and recipient (major)
- Quantified as $2^x (2, \ldots 8\ldots 64\ldots 512\ldots)$
**Blood type switch post HPCT**

<table>
<thead>
<tr>
<th>Blood Group Recipient</th>
<th>Blood Group Donor</th>
<th>Mismatch</th>
<th>Blood type Post Engraftment RBC</th>
<th>Blood type Post Engraftment Serum</th>
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<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>Compatible</td>
<td>O</td>
<td>anti-A, anti-B</td>
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<tr>
<td>O</td>
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<td>Major</td>
<td>A</td>
<td>anti-B</td>
</tr>
<tr>
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<td>Minor</td>
<td>O</td>
<td>anti-B</td>
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<tr>
<td>A</td>
<td>B</td>
<td>Major/Minor</td>
<td>B</td>
<td>anti-B</td>
</tr>
</tbody>
</table>
Transfusion Support ABO-I Tx

- Consider both donor and recipient type
- Donor-compatible plasma and platelets are used to avoid isoagglutinins directed against donor RBCs and to avoid delayed red cell engraftment
- Recipient compatible plasma and pltts to avoid hemolysis
- Donor and recipient compatible RBCs
### Transfusion Support ABO Incompatible HPC Transplants

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>Phase I</th>
<th>←</th>
<th>Phase II</th>
<th>→</th>
<th>Phase III</th>
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<td>A,AB</td>
</tr>
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<td>B</td>
<td>Recipient</td>
<td>O</td>
<td>B</td>
<td>AB;A;O</td>
<td>B;AB</td>
</tr>
<tr>
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<td>AB</td>
<td>Recipient</td>
<td>O</td>
<td>AB</td>
<td>A;B;O</td>
<td>AB</td>
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<td>Recipient</td>
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<td>A</td>
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<td>A;AB</td>
</tr>
<tr>
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<td>B</td>
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<td>AB</td>
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<td>AB</td>
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<td>AB</td>
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<td>AB</td>
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<td>AB</td>
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</table>
New Proposal- Priority: Protecting Engraftment in Phase II

O’Donghaile D et al, Transfusion, 2012
## Clinical Outcomes of ABO-Incompatible Transplants

<table>
<thead>
<tr>
<th>May Cause:</th>
<th>Can Cause:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Delayed neutrophil or platelet engraftment?</td>
<td>• Passenger Lymphocyte Syndrome - Minor</td>
</tr>
<tr>
<td>• Increased risk of GVHD or rejection?</td>
<td>• Pure red cell aplasia (PRCA) - Major</td>
</tr>
<tr>
<td>• Decreased Survival?</td>
<td>• Immediate or delayed hemolysis</td>
</tr>
</tbody>
</table>

Fig 1. ABO incompatibility and overall survival in Kaplan-Meier analysis. ABO-matched (M), minor mismatched (MI), major mismatched (MA), and bidirectional mismatched (BD). HSCT, hematopoietic stem cell transplantation.

Hematopoietic Progenitor Cell Transplantation

Specific Transfusion Strategies

- Avoid alloimmunization (leukoreduced)
- Prevent CMV infection (CMV safe/ CMV seronegative products)
- Prevent TA-GVHD (irradiated)
- Prevent complications of ABO incompatibility
Stem cell infusion reactions
Stem Cell Infusion Reactions

- Reported rates of mild reactions 20-30%
- Reported rates of severe reactions <1%
- Incidence varies depending on the type of product infused:
  - BM, PBSC, or cord
  - Autologous versus allogeneic
  - Fresh or thawed
Reactions similar to all blood components

- Bacterial contamination/Sepsis
- TACO/volume overload
- Allergic
- Febrile non-hemolytic
- Hemolytic
- TRALI
Reactions unique to stem cell products

- DMSO reactions
- Granulocyte reactions
Padley *et al* conducted a large study, reviewing the microbiology data from 7,233 HPC products - 1.6% of products had at least one positive culture result. Skin flora and environmental organisms are the predominant bacteria, mostly coagulase negative staphylococci. The clinical transplant team must be immediately notified, so that a collaborative discussion on the fate of the product can occur. Factors to consider:
- Pathogenicity of the organism, gram negative rods raise particular concern
- Time to growth and the number of positive cultures
- Stage of the patient’s treatment
If proceeding with the infusion of a culture positive HPC product, prophylactic antibiotic therapy may be administered.
Volume Overload:

- Incidence: unknown
  - may be higher than that seen with red blood cell transfusion given the large volume of the transplant, which is often greater than a liter in bone marrow transplant.

- Many HSC transplant recipients are at a higher risk due to their age or renal/cardiac comorbidities and often require transfusion support for thrombocytopenia or anemia during conditioning, so they may already be in positive fluid balance.

- A careful assessment of the patient’s volume status should be performed prior to infusion of the HPCs. HPCs should always be infused slowly with close monitoring.

Preventing Volume Overload

- Splitting of large volume HPC product with infusion on two consecutive days and diuresis in between

- Volume reduction of the product
Allergic reactions

- Manifest in a similar way to allergic transfusion reactions.
- Allergen may be a protein in the allogeneic donor’s plasma or a product added during processing, such as dextran.
- Allergic reactions can often be prevented by premedication with antihistamines, steroids, or a combination of both.
Adverse reactions after infusion of cryopreserved Stem Cells

Contributing factors:

- DMSO
- Post thaw cell aggregation and dead cell debris
- Lysis of RBCs, with release of Hb, electrolytes and membrane fragments
- TNC content and volume of cell suspension.
- Low temperature of infused products
- Electrolyte imbalance
- Premeds such as antihistamines used to neutralize DMSO but can cause bradycardia at the same time
Reactions related to cryopreserved/thawed products

- DMSO (Dimethyl sulfoxide)
  - 10%, osmotically active cryoprotectant
  - <1 g/kg DMSO per recipient body weight per 24 hour period
    (<1 ml/kg)
- DMSO reactions related to histamine release and mast cell degranulation
- May also cause hemolysis and negative chronotropic effects on cardiac tissue
DMSO reactions

- Mild
  - Headache
  - Nausea
  - Shivering
  - Garlic or sweet corn taste
  - Pain at IV insertion

- Severe
  - Cardiovascular: Heart block, hypotension, hypertension, cardiac arrest
  - Neurologic: amnesia, encephalopathy, seizure
  - Pulmonary
  - Hemolysis
Figure 1  Mean centre incidence of DMSO toxicity by DMSO reduction strategy. Error bars show standard errors.

Windrum P Bone Marrow Transplantation, 2005;36, 601-603.
Adverse reactions due to granulocytes in the product
Granulocytes in stem cell infusion

- Granulocytes, both viable and dead cells and cellular debris (membranes, granule contents and cytokines) can cause infusion related toxicities.
- Reactions similar to DMSO
- Thawed/washed stem cell infusions also can cause severe reactions. These are considered to be due to high granulocyte content in the product
- Less than $1.63 \times 10^9$ TNC/kg/day?
Reducing the Infusional Side Effects of Cryopreserved HSC Products

- Premedication with antihistamine, steroids
- Hydration
- Slowing the infusion rate
- Infusing aliquots several hours apart
- Infusing over 2 days
- Washing the HSC product
- Using lower concentration of DMSO during cryopreservation
Documentation and Reporting

- AABB and Foundation of Accreditation of Cellular Therapy (FACT) require a process for the detection, reporting, evaluation, and documentation of adverse events related to stem cell infusion.
- Severe adverse events should be reported to the laboratory immediately.
- Events should be summarized and monitored, through committees such as Transfusion committee or Transplant Quality committees.
Engraftment Failure
Causes of Engraftment Failure

Clinical

- Relapse
- HLA Ab (DSA) or Isoagglutinins
- Conditioning regimen (non-myeloablative)
- Viruses (CMV, Parvo)
- Drugs that damage the BM post tx (Cotrimoxazole and Ganciclovir)
- Low B12 and folate levels
- Insufficient cell dose (inadequate mobilization)

Laboratory

- Insufficient Cell Dose (miscalculation)
- Damage to cells during collection
- Damage to the cells during storage
- Damage to cells during manipulation or cryopreservation
- Expired products used in processing
Laboratory approach to engraftment failure

- Review all initial processing and calculations
- Thaw a cryovial (cryopreserved sample from the initial product) and perform re-characterization
  - WBC
  - Viability (expected to be lower)
  - CFUs
- Confirm no reagents were recalled/expired
Suggested reading

A review of transfusion practice before, during, and after hematopoietic progenitor cell transplantation

James L. Gajewski, Viviana V. Johnson, S. Gerald Sandler, Antoine Sayegh, and Thomas R. Klumpp

Blood. 2008 October 15; 112(8): 3036–3047

