Is it time to re-evaluate the gold standards in blood banking?

Jerry A. Holmberg, MT(ASCP)SBB, Ph.D.
April 24, 2013
2013 California Blood Bank Society
Agenda

- Current status of transfusions in the U.S.
- Traditional serological typing methods
- Red cell genotyping as a supplemental method
- Regulatory considerations
- Recent Blood Product Advisory Committee discussion
Disclaimer

- All blood group genotyping tests commercially available in the US and Canada are for research use only. Not for use in diagnostic procedures.
## Transfusions/1,000 Population Comparison in Selected Countries

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>England</th>
<th>Australia</th>
<th>Denmark</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recipient Age</strong></td>
<td>&lt;41 18.8% 41-65 27.8% &gt;65 53.3%</td>
<td>&lt;40 14.4% 40-70 38.4% &gt;70 47.2%</td>
<td>&lt;40 15.4% 40-70 36.7% &gt;70 47.9%</td>
<td>&lt;39 9.4% 40-59 18.2% &gt;60 72.4%</td>
<td>&lt;39 9.8% 40-59 15.1% &gt;60 75.2%</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>M 48.5% F 51.5%</td>
<td>M 50.4% F 49.6</td>
<td>M 52.5% F 47.5%</td>
<td>M 53.2% F 46.8%</td>
<td>M 52.9% F 47.1%</td>
</tr>
</tbody>
</table>

2009 National Blood Collection and Utilization Survey
Kamper-Jorgensen Transfusion 2009; 49:888-894
Cobain Transfusion Medicine 2007; 17, 10-15
2009 HHS Blood Collection and Utilization Survey of RBC Use by Hospital Service

- General Medicine: 28%
- General Surgery: 11%
- ICU: 11%
- Hem/Onc: 15%
- OB/GYN: 2%
- Transplant: 1%
- Trauma/ER: 9%
- Neph/Dialysis: 2%
- Ped/Neonates: 2%
- Orthopedic Surgery: 6%
- Cardiac Surgery: 7%
- Other: 6%

2009 Health and Human Services’ Blood Collection and Utilization Survey
Transfusion Risk

Risk Assessment

Risk Management

Risk Communication
Challenges in Transfusion Medicine


Notes: TRALI is transfusion related acute lung injury, which occurs within 6 hours of a transfusion. HTR is hemolytic transfusion reaction, which results in destruction of red cells and is usually caused by incompatibility. TACO is transfusion associated circulatory overload and is characterized by a sharp increase in blood pressure.
Challenges in Transfusion Science

- **RBC Alloimmunization: lessons from sickle cell disease**
  - Alloimmunization in general public is 0.5-1.5%
  - Alloimmunization in individuals receiving 3 or more units is 8.4%
  - A review of 12 publications found a mean rate of 25% in SCD

- **RBC Alloimmunization in SCD prevalence in 2010**
  - Editorial (TRANSFUSION 2013;53:692-695.)
  - Drs Treml and King advocates for genotype matching and leads to the conclusion that alloimmunization rates remain high partially to transfusions at institutions not providing extended matching
Challenges in Transfusion Science

- **RBC Alloimmunization in transfused patients with myelodysplastic syndrome or chronic myelomonocytic leukemia**
  - Alloimmunization occurs in 15% of MDS and CMML
  - Alloimmunization mostly involves Rh system and Kell
  - Antigen matching should include Kell and CcEe


- **Immunohematologic and patient safety benefit of a centralized transfusion database**
  - Puget Sound advocates for universal transfusion records since many patients bounce from hospital to hospital

The Future

- Are we moving towards more personalized transfusion medicine?
  - Impact on collections?
  - Impact on testing?
  - Impact on data management?
  - Impact on outcomes?
Agenda

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Scientific Overview

Blood Group Antigens

### Antigen Function

<table>
<thead>
<tr>
<th>CARBOHYDRATES</th>
<th>ADHESION MOLECULES</th>
<th>TRANSPORTERS AND CHANNELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>LW</td>
<td>RH</td>
</tr>
<tr>
<td>P1PK</td>
<td>XG</td>
<td>RHAG</td>
</tr>
<tr>
<td>LE</td>
<td>FY</td>
<td>JK</td>
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<tr>
<td>GLOB</td>
<td>LU</td>
<td>DI</td>
</tr>
<tr>
<td></td>
<td>OK</td>
<td>LAN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>COMPLEMENT REGULATION</th>
<th>ENZYMES</th>
<th>STRUCTURAL OR UNKNOWN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH/RG</td>
<td>KEL</td>
<td>MNS</td>
</tr>
<tr>
<td>CROM</td>
<td>YT</td>
<td>GE</td>
</tr>
<tr>
<td>KN</td>
<td></td>
<td>DO</td>
</tr>
</tbody>
</table>
Traditional Serological Testing Methods

- Limitations of serology to detect antigens in some cases
  - Recent transfusion (mixed field)
  - Warm auto-antibody
  - Antisera
    - Limited availability of some rare antisera
    - Quality

- Weak expression of some RBC antigens

- Impractical to screen large numbers of donors for all potential antigens
  - Typing for rare antigens is often not performed
  - Potential allo-immunization or reactions

- Can be time consuming and costly
4.8.4. Where there is a discrepancy in reaction strength between different anti-D reagents, or where the reagent fails to give a clear-cut strong positive reaction, a decision to investigate further needs to be made based on whether the development of anti-D is likely to cause clinical problems.

7.9. Females of child-bearing potential

- 7.9.1. Females of child-bearing potential should receive K negative red cells unless they are unavailable in an emergency (Lee & de Silva, 2004; BCSH, 2006a).

7.16. Foetal transfusions

- 7.16.2. D negative, K negative (and further antigen negative where appropriate) units should be crossmatched against the maternal plasma by IAT if the maternal plasma contains red cell antibodies of likely clinical significance.

1. Guidelines for the Blood Transfusion Services in the UK 2012
4.1.4. Interpretation of D grouping has become more complex, with the increase in variety of monoclonal reagents, and molecular testing. The historical distinction between weak and partial D, based on whether the individual is able to make anti-D, has become blurred and a new algorithm is included in Fig. 1.
European Perspective

British Committee for Standards in Haematology

Fig. 1. Reporting of D typing anomalies and selection of red cells.


Guidelines for the Blood Transfusion Services in the UK 2012)
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Bloodgen Consortium

Amsterdam, Netherlands
Rotterdam, Netherlands
Lund, Sweden
Ulm, Germany
Prague, Czech Republic
Bristol, UK (UWE and BITS)
Derio, Spain

Banc de Sang i Teixits

Courtesy of Dr. Nuria Nogues, Barcelona
Genotyping for RBC Antigens

- Case for genotyping
  - Basis for predicting phenotypes from genotyping results
    - 324 blood group antigens recognized
    - 33 blood group systems / 40 unassigned antigens
  - Most blood group polymorphisms are now understood

- Potential clinical application
  - Determination of blood type in transfused patients
  - Patients with “allo” or “auto” antibodies
    - warm autoantibodies
    - positive direct antiglobulin test
  - Resolution of phenotype discrepancies
Potential Clinical Application of RBC Molecular

- Detection of rare but significant antigens
- Typing for antigens when antisera are not available
- Identification of a fetus at risk for HDFN
  - Determination of parental zygosity
  - Typing of fetal blood
- Mass screening of donors for antigen-negative blood
- Identification of null and novel alleles

Courtesy of Dr. Nuria Nogues, Barcelona
German Consensus on Blood Group Genotyping

- In 2000 a German consensus statement was developed on the use of blood group genotyping and its potential application in clinical situations (Legler et al. Infusionsther Transfusionmed 2000; 27: 215-16)

- In fetus from amniotic fluid or trophoblastic cells (chorionic villi)

- In multiply transfused patients, if standard serology fails

- In case of auto and allo-immunohemolytic anemia, if standard serology fails

- For weak D types and other variant RH alleles, if serology is inconclusive
Fetal Blood Group Typing in Europe

- Prior to 2001 the usual source of fetal DNA was a sample of amniotic fluid or chorionic villi.

- Cell-free fetal DNA is detectable in the blood of pregnant women → amount increasing throughout gestation.

- Fetal RhD type can be predicted reliably from the fetal DNA in the plasma of D neg pregnant women from beginning of 2nd trimester.

Courtesy of Dr. Nuria Nogues, Barcelona
Non-invasive fetal blood group typing from maternal plasma cell-free fetal DNA is now a clinical reality.

Offered by many laboratories in Europe, to identify the fetus not at risk of HDFN.

Different assays are currently used for reliable genotyping of D, C, c, E and K by quantitative real-time PCR techniques.

Courtesy of Dr. Nuria Nogues, Barcelona
Fetal *RHD* typing: Application to all D neg pregnant women

*European Experience*

- Trials of high-throughput methods have demonstrated that accurate fetal D testing in all D- pregnant women is feasible.

- Fetal *RHD* typing to target antenatal anti-D prophylaxis already introduced in Denmark (2010) and the Netherlands (2011).

**ORIGINALE ARTICLE**

Report of the first nationally implemented clinical routine screening for fetal *RHD* in D- pregnant women to ascertain the requirement for antenatal RhD prophylaxis

*Frederik Bach Clausen, Mette Christiansen, Rudi Steffensen, Steffen Jørgensen, Christian Nielsen, Marianne Antonius Jakobsen, Rikke Dyhrberg Madsen, Karina Jensen, Grethe Risum Krog, Klaus Rieneck, Ulrik Sprogøe, Keld Mikkelsen Homburg, Niels Grunnet, and Morten Hanefeld Dziegieł*

**TRANSFUSION** Volume 52, April 2012
Blood Donors Molecular Typing

Barcelona Blood Center Experience

Focused on specific groups of donors:

- Extensive genotyping of blood donors from immigrant populations

  ⇒ Aim: Identify blood donors expressing low-incidence antigens (Di\(^{a}\), Js\(^{a}\), Mi\(^{a}\), Co\(^{b}\), Kp\(^{a}\)) or donors with rare phenotypes: Di(a+b), Co(a-b+), Fy(a-b-), HPA-1(a-b+)

  ⇒ Utility:

  - For transfusion (rare phenotype blood units)
  - RBC panel units (Antibody identification)
  - Diagnostic (reagent cells)

Courtesy of Dr. Nuria Nogues, Barcelona
Blood Donors Molecular Typing

Barcelona Blood Center Experience

- **RHD** Genotyping of D negative blood donors with RhC and/or RhE positive

  ⇒ Utility:
  
  - Quality control of D negative units
  - Estimate the real incidence of Del units ⇒ 1.6%

- Used the BLOODchip Reference platform

  ⇒ Total of 2,200 blood donors extensively genotyped

Courtesy of Dr. Nuria Nogues, Barcelona
Blood Donors Molecular Typing

Finnish Red Cross Blood Service Experience

- Finnish Red Cross Blood Service experience

Routine genotyping of blood donors with ID-Core+ since September 2012

Criteria
- blood group A or O, K neg
- previous donation within a year
- rare donors, ethnic minorities

Courtesy of Dr. Nuria Nogues, Barcelona
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Traditional Serological Testing Methods

- **Regulatory Process for Serology**
  - Biologics License Application (BLA)
  - Applicable regulations
    - 21 CFR 600-660
    - 21 CFR Part 809 10 (Labeling)
    - 21 CFR Part 820 (Quality System Regulations)

- **Definitions:**
  - Biological Product – 21 CFR Part 600.3 (h)
  - Blood Grouping Reagent - 21 CFR Part 660, Subpart C
  - Reagent Red Blood Cells – 21 CFR Part 660, Subpart D
  - Anti-Human Globulin – 21 CFR Part 660, Subpart F
Traditional Serological Testing Methods

- Hemagglutination assays (HA) defined immunohematology
  - Over 300 blood group antigens recognized

- Reagent Approvals - FDA
  - Polyclonal antisera
  - Monoclonal antisera
  - Anti-Human Globulin (AHG)
  - Panel or screening cells with known antigens
Regulatory Consideration

- High degree of accuracy has been reported
- FDA established molecular testing laboratory to created DNA reference panels
- No licensed or approved molecular device for RBC genotyping

Pathway
- Investigational device exemption (IDE)
- Premarket approval (PMA)
  - Assurance of safety and effectiveness
  - Scientific evidence
  - 180-day review clock
RBC genotyping promises significant technical and clinical benefits for transfusion medicine

Assay systems for RBC genotyping will be reviewed under the PMA pathway

FDA currently considers that:
- In-house validation of RBC genotyping systems will require panels of specimens pedigreed by gene sequencing of the donor
- Candidate technology should be defined within review process to permit appropriate labeling, limitations, and comparison to serology

Guidance and policy will be needed

Dr. Epstein, Director of CBRR, Sept 14, 2012
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Blood Product Advisory Committee

Supports integral labeling with historic antigen type results

- December 2012, BPAC asked to comment on
  - Reporting based on historical RBC typing results from two donations
  - Validated process to confirm donor identification and accurate linkage to historical data
  - Confirmation of relevant negative results on the current unit prior to transfusion, when feasible to mitigate risk from historical data
  - Would BPAC’s response to the first questions vary if serology or molecular testing, or both were performed.

- Committee supported historical data as part of the label and their responses would not vary whether the test was serologic or molecular
Thank you

The secret to complex serology is **in the genes**