ROLE OF MOLECULAR GENOTYPING IN TRANSFUSION MEDICINE

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OBJECTIVES

• Discuss mechanisms of genetic diversity and the molecular bases associated with blood group antigens.

• Describe applications of molecular typing for antigen prediction in transfusion and prenatal settings.

• Describe some instances where RBC and DNA type may not agree.

• Our Experience.
A blood group antigen is a variant form of a **protein** or **carbohydrate** on the outer surface of a RBC that is identified when an immune response (alloantibody) is detected by hemagglutination in the serum of a transfused patient or pregnant woman.

Blood group antigens are inherited, polymorphic, structural characteristics located on proteins, glycoproteins, or glycolipids on the outer surface of the RBC membrane.
• Serological methods remain the **Gold Standard** for quick, routine ABO typing, RHD typing and antibody screening.

• Hemagglutination and other serologic methods have been **proven and well understood** for many years.

• *Molecular* immunohematology is rapidly emerging as a critical tool to **complement serology** with the potential to improve lab productivity and enhance patient safety.
Blood grouping in the 21st century

Already many advances in DNA technology
FROM DNA TO BLOOD GROUPS
The Language of Genes

- DNA: nucleic acid composed of nucleotide bases, a sugar (deoxyribose), and phosphate groups.

- The nucleotide bases are
  1. Purines [A], [G]
  2. Pyrimidines [T], [C]
Some molecular events giving rise to Blood Group Antigens

- **Single Nucleotide Polymorphisms (SNP’s)**
  - Kell, Fy, Jk etc
- **Inactivating mutations & Deletions**
  - Gene, Exon, Nucleotide
  - Null phenotypes
- **Insertion**
- **Single crossover**
- **Gene conversion**

\[ \text{MNS Partial D} \]
Techniques Used to Predict a Blood Group Antigen

1. PCR-restriction fragment length polymorphism (RFLP),
2. Allele-specific (AS)-PCR as single or multiplex assay,
3. Real-time quantitative PCR (Q-PCR; RQ-PCR),
4. Sequencing,
5. Microarray technology.
BEADCHIP™

Application of microbeads of different colours carrying different oligonucleotide probes

Identified by fluorescent scanning on a solid phase

Alleles distinguished by DNA extension
Will molecular genetics replace serological methods for blood grouping in the future?

YES for many tests
• ABO & Rh - complex molecular background
• Serological tests - quick, easy, cheap, accurate
• Important clinical implications of getting it wrong!
APPLICATIONS IN TRANSFUSION MEDICINE
- Limited availability of rare anti-sera
- Lack of regulated, commercial supply for some anti-sera

- Hemagglutination-based methods can limit size of donor pool and depth of antigen information

- Test interpretation sometimes complicated by weakly reactive sera
- DAT+, WAA patients must be characterized with complex methods
- Multiply and recently transfused patients are difficult to phenotype

Source: (1) Reid, “Applications and Experience with PCR-Based Assays to Predict Blood Group Antigens”, Transfusion Medicine and Hemotherapy, 2009;36:168–178
Help establish a larger donor database with greater antigen content at lower costs for labor and supplies

Utilizing high throughput HEA BeadChips to conduct 96 tests across 38 antigens and phenotypic variants in less than 5 hours

Streamlining reference lab operations by informing approach to complex serological work-ups (e.g., DAT+, high incidence antigen, etc.) with genotype information

Source: (1) Reid, “Applications and Experience with PCR-Based Assays to Predict Blood Group Antigens”, Transfusion Medicine and Hemotherapy, 2009;36:168–178
Transfuse red blood cells with extended matching to reduce incidence and outcomes of alloimmunization

An aide in ensuring availability of compatible blood products with aid of high-throughput typing system

Meet the blood supply needs of poly-transfused patient populations requiring antigen-negative units (e.g. thalassemia, sickle cell disease, blood oncologies, kidney disease)

Clinical / patient care benefits

All patients

• Transfuse red blood cells with extended matching to reduce incidence and outcomes of alloimmunization
• An aide in ensuring availability of compatible blood products with aid of high-throughput typing system
• Meet the blood supply needs of poly-transfused patient populations requiring antigen-negative units (e.g. thalassemia, sickle cell disease, blood oncologies, kidney disease)

Poly-transfused patients

• Provide specialized, well-characterized products for high risk procedures (e.g. bone marrow transplant, solid organ transplant)
• Provide genotypically matched products for patients with positive DATs, warm autoantibodies, high incidence antigens

High-risk procedures

Serologically complex patients

Source: (1) Reid, “Applications and Experience with PCR-Based Assays to Predict Blood Group Antigens”. Transfusion Medicine and Hemotherapy. 2009;36.168–178
Applications in the Prenatal Setting: Fetal genotyping for prediction of risk of HDFN

Criteria should be met before obtaining fetal DNA for analyses:

- the mother’s serum contains an IgG antibody of potential clinical significance
- and the father is heterozygous for the gene encoding the antigen of interest or when paternity is in doubt.
- It is helpful to know the ethnic origin and to concurrently test both mother and father
D-negative pregnant woman with anti-D

Valuable to know D type of fetus

Fetus D-positive: at risk
pregnancy should be managed appropriately

Fetus D-negative: not at risk
no need for intervention
Source of fetal DNA

Before 2001
DNA from amniocytes

Amniocentesis:
0.5-1.0% risk of spontaneous abortion
20% risk of transplacental haemorrhage

Better source of fetal DNA required
Fetal DNA in Maternal Plasma

Maternal cell-free DNA

1st trimester: 3% cell-free DNA = fetal
3rd trimester: 6% cell-free DNA = fetal

Excellent source of fetal DNA for fetal *RHD* testing

Non invasive
Cell free fetal DNA for prenatal diagnosis

**Advantages**
- No miscarriage risk
- Potentially earlier test and more acceptable
- Reduced parental anxiety

**Problems**
- Emanates from placenta-? mosaicism
- Unreliable in multiple pregnancy
- In early pregnancy, risks associated with vanishing twin
cffDNA and RHD
Potential in routine antenatal care

Check fetal RHD group

positive

Anti-D at 28 and 40 wks

negative

No Anti D
Other tests on fetal DNA in maternal plasma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Symbol</th>
<th>SNP</th>
<th>Exon/Intron</th>
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<tbody>
<tr>
<td>K</td>
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<td>RHCE</td>
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RQ-PCR with an allele-specific primer
For Transfusion-dependent Patients

- Sickle cell disease
- Thalassemia
- Autoimmune hemolytic anemia
- Aplastic anemia

- Presence of donor RBCs in the patient’s peripheral blood
For Patients Whose RBCs Have a Positive DAT

- **Warm autoantibodies**: Autoantibody present in the serum/plasma may mask the formation of an underlying alloantibody.

- As an aid in the selection of RBCs for heterologous adsorption of the autoantibody.
For Blood Donors

• To predict the antigen type of donor blood both for transfusion and for antibody identification reagent panels. Useful when antibodies are not available or are weakly reactive. eg Dombrock

• Antibodies not known to cause RBC destruction: Ab to Knops blood group system, BUT Are often found

• To test donors to increase the inventory of antigen-negative donors.

• For the resolution of apparent discrepancies, for example, the resolution of ABO typing discrepancies due to ABO subgroups, and for reagent discrepancies
Defining RhD variants: weak D and partial D

WHY?

Is the patient likely to make anti-D? Is IgG anti-D prophylaxis needed?
For Patients and Donors

Detecting **Weakly Expressed** Antigens

- Eg. patient with a weakened expression of the Fyb antigen due to the Fyx phenotype is *unlikely to make antibodies* to transfused Fy(b) RBCs.
Platelet antigen alloimmunization can induce

1. Neonatal alloimmune thrombocytopenia (NAIT)
2. Post-transfusion purpura
3. Platelet transfusion refractoriness

Platelet donors can also be typed and called for donation in case of any of the above conditions. The **HPA typed panel of donors** (registry) is already in place in certain countries.
Human Neutrophil Antigen (HNA) Typing

Neutrophil-specific antibodies are implicated in:

1. Febrile non-haemolytic transfusion reactions
2. Transfusion-related acute lung injury (TRALI)
3. Neonatal alloimmune neutropenia
4. Autoimmune neutropenia
5. Persistence of post-bone marrow transplant neutropenia
6. Transfusion-related alloimmune neutropenia (TRAIN)
7. Drug-induced neutropenia
**Silencing changes**: If a gene is not expressed on his or her RBCs, he or she could produce an antibody if transfused with antigen-positive blood. Eg

- GATA box with FY typing,
- presence of *RHD pseudogene with RHD typing*,
- and exon 5 and intron 5 changes in *GYPB with S typing*. 
Implementation in the Blood Bank

How do we get started?

Where should it be done?

- Reference laboratory i.e. centralised
- Blood donor centre (typing donors)
- Hospital (typing patients) ???

Centralisation important
Who should be doing it?

- **Expert personnel**
  - Understanding applications
  - Understanding blood group genetics
  - Setting up appropriate tests
  - Interpretation of results

How do we get started?
What do you need?

- Facilities for DNA isolation and amplification (clean room)
- IT expertise
- Must be cost effective

How do we get started?
Impact of antigenic exposures and role of molecular blood grouping in enhancing transfusion safety in chronically transfused thalassemics

Raj Nath Makroo, Soma Agrawal, Aakanksha Bhatia, Mohit Chowdhry, and Uday Kumar Thakur

Aim:
To perform molecular blood group genotyping in chronically transfused thalassemia patients and assess the risk of antigenic exposure and incidence of alloimmunization with current transfusion protocols.

Materials and Methods:
Molecular blood group genotyping was performed for 47 chronically transfused thalassemia patients. Their 1-year transfusion records were retrieved to assess the antigenic exposure and the frequency thereof.

Results:
Random selection of ABO and Rh D matched units resulted in 57.7% ± 8.26% chance of Rh and Kell phenotype matching also. 44 patients had received one or more antigenic exposures at least once.

<table>
<thead>
<tr>
<th>Antigenic stimulus</th>
<th>Antigen negative patients</th>
<th>Patients exposed (%)</th>
<th>Average frequency of exposure % (range)</th>
<th>Alloimmunization during study period</th>
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<tbody>
<tr>
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THANK YOU