Applications of Red Cell Genotyping in Serological Problem-Solving

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Molecular vs Serological Phenotypes

- Serological Phenotyping Advantages
  - Detects antigens that are physically on red cells
  - Gold standard
  - Quick single antigen results, from instant to 45 min
  - Most antisera for routine antigen testing are FDA approved

- Serological Phenotyping Disadvantages
  - Limited antisera selection
  - Cost $$$
  - Labor intensive for large scale testing
  - Variability in reactivity
  - Does not detect variants well
  - Not suitable for transfused patients

- Molecular Phenotyping Advantages
  - Detects the presence or absence of genes to predict the phenotype
  - For most platforms, in depth analysis of at least 11 Blood Groups with results for at least 35 antigens and 4 mutations
  - Possible identification of various RH variants like D variants, r’s and other c0 variants
  - Possible identification GATA-1 mutation which may indicate the presence of Fyb antigen on tissue cells and the option of providing Fyb positive RBCs
  - Possible identification of U+ vs. Uvar due to silencing S mutation
  - Possible identification of various high and low incidence antigens not available with routine antiseras
  - Great for large scale screening
  - Testing is not affected by transfusions (except after transfusion of non leukoreduced products)
Molecular vs Serological Phenotypes

- Molecular Phenotyping Disadvantages
  - Longer testing time, at least 6-8 hours to days
  - Not practical for single antigen testing
  - Difficult data management
  - May not always give an accurate phenotype due to gene variants
  - May require more advanced training for data interpretation

Molecular Testing Methods

- 3 Steps are Involved in Most Molecular Techniques
  - Nucleic Acid Extraction: To obtain DNA, RNA template
  - Amplification (PCR): To multiply the gene targets of interest
  - Detection: To identify the present or absents of the targets of interest

Molecular Testing Methods

- Detection of Single Nucleotide Polymorphisms (SNPs) to predict the phenotype
  - Gel Electrophoresis
  - Micro Arrays (1 FDA approved platform)
  - MALDI-TOF mass spectrometer
    - Matrix-assisted laser desorption/ionization (MALDI)
    - time-of-flight mass spectrometer (TOF)
Best Use of Molecular Phenotyping

- **Donor Centers**
  - Larger scale antigen typing with quick turn around time.
  - For most platforms, in depth analysis of at least 11 Blood Groups with results for at least 35 antigens and 4 mutations.
  - Quick identification of missing high incidence antigens like k, Kpb, Jsb, Coa, Lwa, etc. indicating rare units.
  - Identification of Fy* which can be serologically typed as Fyb negative but can cause the sanitization to Fyb antigen.
  - Quick identification of the presence of low incidence antigens like Kpa, Js, Cob, etc. This can help in antibody identification.
  - Now FDA approved with the ability to label units with antigen typing results.

Best Use of Molecular Phenotyping

- **Hospitals/Patients**
  - Sickle cell hemoglobinopathy (require Rh and K match blood)
  - Auto Immune Hemolytic Anemia
  - Complex antibodies
  - Chronically transfused
  - Some obstetrical

Sickle Cell Hemoglobinopathy

- Recommend molecular phenotyping when first diagnosed
- **Why?**
  - The large majority of SCD patients are African American, and are known for various Rh variants and lack of certain high incidence antigen
  - Higher rates of alloimmunization and formation of warm auto antibodies
Sickle Cell Hemoglobinopathy

• Advantage of a molecular phenotype
  – A routine molecular phenotype will give you results for Rh, Kell, Kidd, Duffy, MNS and a combination of other antigens.
  – Ability to identify GATA-1 mutation (silencing Fyb) which may allow you to safely transfuse Fyb positive RBCs to Fyb negative recipient
  – Ability to identify missing high incident antigens like Jsbs and U
  – Ability to identify potential Rh variants like r's or other ce variants

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Sickle Cell Hemoglobinopathy

• Advantage of a molecular phenotype
  – If an Rh variant is suspected, you may need to do addition testing to identify the specific variant in order to evaluate the risk of alloimmunization to high incidence antigens within the Rh system. Should always do an RHD and RHCE analysis to fully identify the variant(s)
  – Having a complete phenotype on file can be useful in antibody identification down the road
  – Can be performed even if the patient was recently transfused

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Auto Immune Hemolytic Anemia

• Recommend molecular phenotyping when first showing signs of development of warm auto/panagglutinin antibodies with anemia
• Why?
  – This patient population is difficult to serologically phenotype because they often have positive DAT IgG test, molecular phenotyping is not affected.
  – A full phenotype can be useful if transfusion of least incompatible blood is needed (recommend full match RBCs)
    • May allow you to extend the time between full workups

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Auto Immune Hemolytic Anemia

- A full phenotype is useful for antibody identification in the presence of a warm auto
- Can help differentiate between true auto antibodies and allo antibodies to high incidence antigens or variant antigen
  - If auto antibodies have an Rh specificity, it could be due to an Rh variant in which case you may be dealing with an allo antibody
  - Example: auto antibodies with e specificity may be allo anti-hb
- Can help in the rule out process or selection of cells for differential adsorption

Complex Antibodies

- Recommend molecular phenotyping when complex antibody or multiple antibodies are present
- Why?
  - Having a full phenotype on hand can be useful in the rule out process or selection of cells.
  - Can help in the identification of less common antibodies like anti-Dob or anti-Ytb.
  - May cause delay in the workup since it may take a day or two to get results back for the molecular phenotype.

Chronically Transfused

- Recommend molecular phenotyping for chronically transfused patients. This can be thalassemia, hypo/aplastic anemia, myelodysplastic syndrome or any condition that require monthly transfusions for a period of time.
- Why?
  - Much like sickle cell disease patients, chronically transfused patients will benefit from a C, E and K match protocol.
  - Although not as much a sickle cell disease patients, chronically transfused patients still have a higher rates of alloimmunization than the general population.
Obstetrical

• Recommend molecular phenotyping for HDFN cases due to Rh D or other clinically significant antibodies.
• Why?
  – Molecular testing can be performed on circulating fetal DNA within the mom’s blood. This can avoid risky amniocentesis or other procedures on the fetus.
  – Molecular testing can tell homozygosity of specific antigens like Rh D and help predict the potential risk to the fetus.

Any questions?

Thank you for your time
And if you can donate blood
please remember to give