EDUCATIONAL COMMENTARY – MOLECULAR TESTING OF BLOOD ANTIGENS

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Learning Outcomes
Upon completion of this exercise, the participant should be able to:

- explain the methodology of beadchip molecular testing in transfusion medicine.
- describe the clinical applications for such testing.
- evaluate the current limitations of molecular testing in transfusion medicine.

A 60-year-old man had shortness of breath and anemia. A specimen was sent to the blood bank in anticipation of a need for transfusion. Results revealed that he had a warm autoantibody. Because he had been transfused before, an autoadsorption was not possible, and a differential adsorption was attempted. This labor-intensive test was repeated several times, but the laboratory was unable to determine if the patient had an underlying alloantibody. A decision was made to transfuse using least-incompatible blood, and a genotype was performed to be prepared for future transfusions, as well as to better understand the risks with the current transfusion.

Another patient was diagnosed with sickle cell disease. The patient was likely to need chronic transfusions, so a genotype was ordered. Along with the patient’s phenotype on file, better-matched blood could be offered, reducing the risk of alloimmunizing the patient.

Background
Serology, the mainstay of blood bank testing, has been in practice for more than 100 years. Serologic testing has shortcomings; it can be subjective and has numerous limitations. Some anti-sera are costly, difficult or impossible to obtain, and has a limited shelf-life.

Molecular techniques are now routinely used in hematopathology and microbiology. With the current genetic understanding of the blood group antigen systems, transfusion medicine has become a rich area for molecular testing. As opposed to serology, which looks at what molecules are present on the surface of a cell, genotyping studies the genes that will determine what molecules will be present. Molecular testing in transfusion medicine has been heralded in publications from *Transfusion* to *Clinical Chemistry* to *Science*. Most blood group antigens are inherited as single nucleotide polymorphisms (SNP), such that a single nucleotide makes the difference between two antigens within the same system. For example, Jk\(^a\) and Jk\(^b\) in the Kidd blood group are differentiated by a substitution of guanine (G) for adenine (A) at position 838 in the DNA sequence. The ABO and Rh blood groups are significantly more
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complicated and will be discussed later; however, for most of the antigen systems, polymerase chain reaction (PCR) can be used to isolate and amplify a portion or the polymorphism of a gene. Examination of the PCR product can determine the genotype of a patient, which usually translates into the phenotype of the patient. Specifically, genotype refers to the alleles at a single gene locus that a person inherits from his or her parents, but the phenotype is the expression of those inherited genes and reflects the biologic activity of the gene.

By early 2000, companies in Europe and the United States endeavored to use molecular means to type blood. Two companies offer such testing: BloodGen, a European company that offers the BloodChip, and its American counterpart, BioArray, which offers the BeadChip.

Technology
BeadChip technology is available in the United States and uses an oligonucleotide primer or probe sequence attached to a spectrally distinguishable bead. The Figure on the next page outlines the steps used in this technology. This DNA sequence is a SNP representing a single blood group antigen, and only one nucleotide is paired with one bead. Thousands of these colorful beads are then attached to a chip and placed on a microscopic slide constituting a library of sorts (that is, prior to the patient testing, a decoder image is taken to know the location of the bead with its unique SNP). Because mature red blood cells (RBCs) have lost their genetic material, DNA is extracted from the patient’s peripheral white blood cells (whole blood drawn into an EDTA tube) or from a buccal swab. The DNA is then amplified by PCR. The PCR products are then digested into multiple, specific, single-stranded DNA fragments that are then exposed to the chip. When the complimentary DNA sequence is present it will anneal to the primer/probe on the bead resulting in elongation of the nucleotide fragment. During the elongation process, fluorescently-labeled nucleotides are inserted. A digital photograph is then taken of the fluorescence pattern and through a decoder image, the system software is then able to determine what DNA sequences are present in the patient sample. By knowing a patient’s genotype, the phenotypic profile of the patient’s RBCs is likewise known, that is, we know whether or not the patient’s RBCs express the antigen in question. In the Human Erythrocyte Antigen (HEA) 1.2 BeadChip test (BioArray, Warren, New Jersey), for example, testing is possible for 32 human erythrocyte antigens (Table I). Additionally, the BioArray system can detect hemoglobin S; however, it is not capable of testing for the more complicated A, B, or D antigens.
**Figure.** Summary outline of the processes used in BioArray BeadChip technology. PCR indicates polymerase chain reaction. (BioArray, Warren, New Jersey)

1. Whole blood sample or buccal swab
2. DNA extraction
3. PCR amplification and processing
4. Hybridization on the BeadChip
5. Strand extention with incorporation of fluorescent nucleotides
6. Imaging
7. Data analysis (genotype-to-phenotype conversion)

Table I may be found on the next page.
TABLE I: Major Antigens Tested for by the HEA 1.2 BeadChip Kit.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>C and c</td>
</tr>
<tr>
<td></td>
<td>E and e</td>
</tr>
<tr>
<td></td>
<td>Vs and V</td>
</tr>
<tr>
<td>Kell</td>
<td>K and k</td>
</tr>
<tr>
<td>Duffy</td>
<td>Fy^a and Fy^b</td>
</tr>
<tr>
<td>Kidd</td>
<td>Jk^a and Jk^b</td>
</tr>
<tr>
<td>MNS</td>
<td>M and N</td>
</tr>
<tr>
<td></td>
<td>S and s</td>
</tr>
<tr>
<td>Lutheran</td>
<td>Lu^a and Lu^b</td>
</tr>
<tr>
<td>Dombrock</td>
<td>Do^a and Do^b</td>
</tr>
<tr>
<td></td>
<td>Jo(a+) and Jo(a-)</td>
</tr>
<tr>
<td></td>
<td>Hy+ and Hy-</td>
</tr>
<tr>
<td>Landsteiner-Wiener</td>
<td>LW^a and LW^b</td>
</tr>
<tr>
<td>Diego</td>
<td>Di^a and Di^b</td>
</tr>
<tr>
<td>Colton</td>
<td>Co^a and Co^b</td>
</tr>
<tr>
<td>Scianna</td>
<td>Sc1 and Sc2</td>
</tr>
</tbody>
</table>

Kit manufactured by BioArray (Warren, New Jersey). Testing for hemoglobin S is also offered. The complete testing profile can be found in the product insert or the company website.

Clinical Applications

Table II lists the clinical situations most favorable for the use of genotyping. The major advantages of genotyping are that the test results are not affected by a recent transfusion, antibodies coating RBCs, or weakened RBC antigen expression. In fact, RBCs are not needed to perform the test. Serologic phenotyping is challenging with a patient who has been recently or chronically transfused because donor RBCs circulate for up to three months. Molecular testing counteracts the challenge because the recipient’s DNA far outnumbers donor DNA present in the white blood cells of the transfused products. It also allows the transfusion service to determine a patient’s extended phenotype and provide phenotypically matched blood thereby preventing alloimmunization against clinically significant antibodies. This may also allow the blood bank staff to hone in on an alloantibody that may be causing a delayed hemolytic transfusion reaction.

Furthermore, molecular testing can be useful in patients with multiple alloantibodies. The genotype can effectively guide the blood bank in the antibody investigation of patients who are serologically complex
and whose results tend to be inconsistent or confusing. The various methods of serologic testing, including tube, gel, and solid phase testing, can lead to discrepancies from the differences in methodologies and reagents.

Patients with autoimmune hemolytic anemia represent another cohort that benefits from genotyping. The presence of panreactive autoantibodies interferes with serology-based phenotyping, and the methods to overcome this are time-consuming and laborious. Although molecular testing cannot eliminate all the challenges created with an autoantibody, it provides the blood bank staff with insight in providing partially or fully phenotypically matched RBCs.

**TABLE II. Clinical Scenarios in Which Genotyping Should be Considered**

- Patients who may receive multiple transfusions (sickle cell, transplant, hemophilia, leukemia)
- Autoimmune hemolytic anemia
- Patients with a positive direct antiglobulin test
- Autoantibodies
- Patients with multiple alloantibodies
- Patients with confusing or conflicting serologic results
- Patients who have had transfusion reactions
- Pregnancy, for mothers who have or are at risk for forming clinically significant antibodies such as in partial or weak D, hemolytic disease of the newborn, or neonatal alloimmune thrombocytopenia
- Fetal typing when the sample is from amniocytes or cell-free DNA

**Limitations**

The major limitations of molecular testing include: time, availability, expense, and correlation between genotype and phenotype. Most laboratories are not equipped to perform this testing at the present time, and specimens must be shipped to reference laboratories. The test can be completed in a matter of hours, but typically the turnaround time is one day. Serology by contrast can be performed in every laboratory, and testing can commence immediately. Even so, these limitations should not be a deterrent for ordering the test that may aid the blood bank for future management of a patient. Since genotyping focuses on known polymorphisms and not sequencing the entire gene, there are several situations in which genotyping does not predict the phenotype. These situations include: new polymorphisms in the coding region that may influence the protein structure; changes in the promoter or enhancer region that change the gene expression, or changes in the epitopes that will influence the posttranslational modification of the epitopes by bacterial enzymes. For example, the BeadChip also tests for silencing mutations for the Duffy and MNS blood groups. Most importantly, genotyping does not give information about what allo- or autoantibodies are present, and this is a critical aspect in transfusion practice, thus serology remains central to this investigation. While serology is relatively inexpensive, molecular testing
can be cost-effective and will likely become more so as the technology and the way to deliver the technology continues to improve. For the BeadChip, the combined price for the DNA extraction and kit is approximately a few hundred dollars.

**Future**

Considerable work remains before genotyping is routinely implemented. But a future in which all donors have their blood genotyped is not an unreasonable expectation. Further, this could lead to a better match for a typed recipient, the so-called individualized transfusion treatment, thus reducing many transfusion complications. Even though we cannot match for every antigen, the blood supply is limited, and time constraints sometimes lead to urgent transfusion, molecular testing has the potential to improve patient care. Such testing is pending approval by the Federal Drug Administration (FDA) as donor units cannot be officially labeled with the genotype information. Transfusion services can choose to accept genotype information; however, FDA-approved serologic testing must be performed for phenotypic confirmation prior to labeling and dispensing blood as antigen negative for a patient.

The genetics behind the ABO and Rh groups is significantly more complicated. While many of the blood group antigens are defined by simple genetic changes, there are currently more than 100 different RhD genes and including the many subgroups of A and B, there are more than 100 genes for the ABO blood group system. Thus testing would need to examine multiple regions of these genes. While some molecular testing is available in Europe, more work is required before we can confidently test for these antigen systems.

Molecular testing can be extended to platelet antigens, a useful tool for patients with platelet refractoriness or cases of neonatal alloimmune thrombocytopenia and posttransfusion purpura. This methodology can also be done with HLA A and B typing; a patient with platelet refractoriness can be offered HLA-matched platelets. More work needs to be done before a more thorough HLA analysis (classes I and II) can be offered for the transplant setting.

Other forms of molecular testing can also play a significant role in fetal and maternal care. For the fetus, genotyping can be of use in patients at risk for hemolytic disease of the fetus or newborn as this allows for Rh zygosity testing in the fetus and parents. Specifically, for women of childbearing age, weak and partial D may be of significant concern. A mother with a weak D will not form antibodies to the D antigen as opposed to a mother with partial D. Thus knowing the difference will influence whether to offer Rh-negative blood and Rh immune globulin. While serology cannot distinguish between the two, techniques using PCR for genotyping the RHD gene can. These techniques are currently only available in reference laboratories.
Educational Commentary – Molecular Testing of Blood Antigens (cont.)

Suggested Readings


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