Molecular Genotyping in Transfusion Medicine
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“Blood-Matching Goes Genetic,” a recent News Focus article in Science (1), illustrates the growing awareness of the impact of the genomics revolution on transfusion medicine and its potential to transform the way blood is selected for transfusion. Antibody-based technology has been the basis for blood group typing since the discovery of the ABO blood groups by Landsteiner and colleagues. With the availability of single-nucleotide polymorphism (SNP)3 genotyping, the blood bank is poised to become one of the primary laboratory disciplines to benefit from the PCR-technology revolution.

DNA-testing methods are applicable to transfusion medicine because the genes encoding blood group antigens and the genetic polymorphisms associated with the antigens are known. Similar to many disease markers, most of the antigens in blood groups result from SNPs inherited in a straightforward Mendelian manner. SNPs account for much of human genome diversity, and the ability to link SNPs to disease or disease risk, and even to individual variation in drug responses, makes SNP profiling a technology likely to contribute to fulfilling the promise of personalized medicine. The appreciation of this potential has fueled the development of high-throughput SNP-genotyping platforms, which are currently being applied in the prediction of blood group antigens (2). The use of such platforms enables rapid, cost-effective screening for multiple clinically significant blood groups in a single assay. The field of transfusion medicine can now consider individualized transfusion treatment, i.e., matching donors with patients at multiple blood group loci in addition to the ABO and RhD systems. This approach holds promise for reducing, and potentially eliminating, alloimmunization.

Expanded Typing

SNP typing allows the evaluation of blood group antigens in circumstances in which it is not possible with antibody-based methods. For example, in patients receiving chronic or massive transfusions, the presence of donor red blood cells (RBCs) makes typing inaccurate. In patients with autoimmune hemolytic anemia or with RBCs coated with immunoglobulin in the absence of hemolysis, the presence of bound IgG often invalidates typing results in the indirect antiglobulin test. DNA-based methods overcome these limitations. Because regions of genes common to all alleles are targeted, the minor amounts of donor DNA that might be present are outcompeted by patient DNA during the PCR. Hence, the results obtained from transfused patients are accurate. Accurate typing of patients who receive massive transfusions of non–leukocyte-reduced blood could also be ensured by testing DNA isolated from a buccal swab.

Antibody-based typing reagents are not available for all of the clinically significant blood group antigens, and this fact can complicate finding compatible blood for patients with antibodies to antigens such as those in the Dombrock, Colton, Kell (Js, Kpg), and Diego systems. Although the US Food and Drug Administration has not yet approved SNP-typing methods for labeling donor units, these methods are valuable for finding compatible units.

Prenatal Testing

Antigen typing with DNA-based assays has particular value in pregnancy if the mother has antibodies to erythrocyte or platelet antigens. Determining the paternal allele inherited by the fetus is key to assessing the risk for hemolytic disease of the fetus and newborn or for neonatal alloimmune thrombocytopenia. In prenatal practice, such determinations most often involve identifying RhD or platelet HPA-1a (PfA1) antigens, which are usually identified serologically. If the father tests positive in antibody-based typing, the paternal gene copy number (i.e., homo- or heterozygosity) is determined with DNA methods. If the father is homozygous for the gene, all of the children will be positive, and monitoring of the pregnancy is required. If the father has a deletion in the RHD gene (Rh blood
group, D antigen) or has an inactive \textit{RHD} gene (or is HPA-1a/1b when assessing the risk for neonatal allo-immune thrombocytopenia), the fetus may be antigen negative and not at risk. Fetal status is determined by testing fetal DNA from cells obtained by amniocentesis or by testing cell-free, fetal-derived DNA present in maternal plasma at 5 weeks gestation (or later) \cite{3}. If the fetus is antigen negative, the mother and fetus need not undergo invasive, costly monitoring or receive immune-modulating agents.

**Maternal D Determination**

D typing of RBCs can be complicated in the approximately 2% of individuals who have a variant \textit{RHD} gene that encodes a reduced concentration of D antigen (weak D) or antigen with missing D epitopes (partial D). Different antibodies present in manufacturers’ reagent sets can react differently with these RBCs, and interpretation of D type can depend on the method used (i.e., use of tubes, solid phase, gel, or automated analyzers). The distinction between weak D and partial D is a concern in women of childbearing age. Those with partial D, but not usually those with weak D, may make anti-D antibody and should be considered D negative for transfusion and as candidates for Rh immune globulin. Currently available serologic reagents cannot distinguish the 2 D types, but \textit{RHD} genotyping can.

**Considerations**

SNP-based typing for ABO is more challenging because group O is produced by a large number of different mutations that inactivate the A or B transferase enzymes. Many different subgroups of A and B exist, and >100 alleles have been reported. These facts will require testing platforms that sample numerous regions of the genes. The risk of ABO mistyping if the sample has a previously unknown inactivating allele may be unacceptable when selecting blood for transfusion. At present, ABO typing with multiple SNPs or exonspecific gene sequencing is valuable for resolving ABO serologic discrepancies or for typing cell lines and tissues when RBCs are unavailable.

SNP typing predicts the phenotype, but DNA typing may not reflect the true RBC phenotype in some instances. This situation can occur if an unrecognized polymorphism not targeted by the SNP system suppresses or inactivates the expression of the gene, thus producing a false positive. Indeed, for Duffy (Fy) and glycoporphin B (Ss) groups, the detection of silencing mutations is also needed for accurate typing.

Importantly, SNP typing cannot replace antibody screening and identification to determine if the patient is alloimmunized and, if so, to which antigens.

**Alloimmunization Risk and Inventory Management**

The application of molecular genotyping to transfusion medicine has the potential to dramatically change blood bank testing by enabling the electronic selection of donor units that have been antigen-matched for recipients at multiple blood group loci. This approach could improve patient care and transfusion outcomes by reducing alloimmunization. It will not be possible, however, to match all 29 blood group systems (nor would it be prudent, because not all systems are clinically significant), and inventory limitations preclude providing exactly matched units to every patient requiring RBC transfusion. Focusing on the 5 primary antigen systems [Rh (Cc, Ee), Kell, Kidd (Jka/b), Duffy (Fya/b), and Ss] and patient prioritization would be needed so that patients receive extended-matched units according to alloimmunization risk.

Two issues to consider in patient prioritization for extended matching are the likelihood of developing antibody if the patient is exposed to the corresponding foreign antigen and the clinical significance of the antibody, because some antibodies are more likely to cause serious hemolytic reactions to transfusions. RBC alloimmunization is manageable for many patients not facing long-term transfusion support. Although alloimmunization adds complexity to future evaluations by blood bank laboratories and may complicate emergency transfusions, serious complications are unusual. In contrast, alloantibodies can have serious consequences for sickle cell patients and pregnant women. Patients with sickle cell disease have compromised RBC survival and are anemic. Hemolysis due to an alloantibody could cause serious anemia and trigger a sickle cell crisis. Complications from alloantibodies in pregnant women can range from serious or fatal hemolytic disease of the fetus or newborn to costly monitoring of a high-risk pregnancy.

Statistical analyses suggest that approximately 13% of patients are at risk of forming alloantibodies. Currently, the patients who are more likely to develop antibodies cannot be prospectively identified, but multiply transfused patients with sickle cell anemia and any patient who has made one alloantibody are at substantially increased risk of making additional antibodies \cite{4}.

Although providing extended-matched RBC units is unlikely for all patients, it will soon become feasible for many. As genotyping technology advances, as blood inventory—management systems improve, and as recruitment efforts focus on minority donors, more pa-
tients will be able to receive extended-matched transfusions. An initial step may be to provide some degree of extended-matched RBC units for sickle cell patients and patients who have made an alloantibody. Female patients of childbearing potential would also be better served by receiving K- and c-matched units (5).

Alloimmunization may never be prevented in all patients. There are >100 potentially antigenic polymorphisms, and not all can be detected with SNP-typing systems. Turnaround time is also a consideration. Current multiplex PCR systems require about 7 h, from DNA extraction of the sample to readout. Although this time may be substantially reduced in the future, patients urgently requiring transfusion will be initially unable to take advantage of an extended-matched capability.

Conclusions

SNP typing is a powerful adjunct to serologic testing and is superior for typing transfused patients, paternal zygosity determination, noninvasive fetal typing, and antigen matching of patients. Recent efforts have focused on determining whether known genetic polymorphisms in many different blood group systems accurately reflect the RBC antigen phenotype for all individuals in different ethnic groups. Significant progress has been made in validating gene targets and in investigating and explaining discrepancies between phenotype and genotype. Assays for blood antigens encoded by single SNPs are highly reproducible and correlate with RBC phenotype. Until recently, SNP typing for transfusion medicine has been limited primarily to reference laboratory environments; however, with the development of high-throughput platforms, testing is poised to move into the mainstream to revolutionize the provision of antigen-negative blood and RBC units antigen-matched at multiple blood group loci to recipients. The challenge for the next decade lies in integrating such testing into the blood bank environment, standardizing methods, obtaining Food and Drug Administration approval for labeling donor units, and enhancing information systems to incorporate and use this new information effectively.

References