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Pediatric Section

Red Blood Cell Antigen Genotyping for Sickle Cell Disease, Thalassemia, and Other Transfusion Complications

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ABSTRACT

Since the discovery of the ABO blood group in the early 20th century, more than 300 blood group antigens have been categorized among 35 blood group systems. The molecular basis for most blood group antigens has been determined and demonstrates tremendous genetic diversity, particularly in the ABO and Rh systems. Several blood group genotyping assays have been developed, and 1 platform has been approved by the Food and Drug Administration as a "test of record," such that no phenotype confirmation with antisera is required. DNA-based red blood cell (RBC) phenotyping can overcome certain limitations of hemagglutination assays and is beneficial in many transfusion settings. Genotyping can be used to determine RBC antigen phenotypes in patients recently transfused or with interfering allo- or autoantibodies, to resolve discrepant serologic typing, and/or when typing antisera are not readily available. Molecular RBC antigen typing can facilitate complex antibody evaluations and guide RBC selection for patients with sickle cell disease (SCD), thalassemia, and autoimmune hemolytic anemia. High-resolution *RH* genotyping can identify variant *RHD* and *RHCE* in patients with SCD, which have been associated with alloimmunization. In the future, broader access to cost-efficient, high-resolution RBC genotyping technology for both patient and donor populations may be transformative for the field of transfusion medicine.

Contents

Genetic characterization of blood group antigens has demonstrated significant diversity among populations. Genomic data are increasingly incorporated into standard clinical care, and molecular methods to predict red blood cell (RBC) antigen phenotypes have improved our approach to transfusion practice. Hemagglutination using RBCs and blood group antigen-specific antisera is a simple and robust method for RBC antigen typing of blood donors and patients, but has some limitations for which DNA-based testing provides an alternative. These include

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extended RBC phenotype determination in patients recently transfused or with interfering allo- or autoantibodies, discrepant serologic typing results and/or when typing antisera are not readily available, paternal RhD zygosity testing for pregnancies at risk for RhD hemolytic disease of the fetus and newborn (HDFN), and prenatal determination of fetal blood group antigen status in allosensitized mothers at risk of HDFN (Table) [1]. Red blood cell genotyping is also an efficient method for donor centers to identify RBC units with rare or uncommon antigen phenotypes, or simply to meet demands for antigen-negative units (Table) [2]. In particular, maintenance of large pools of African American blood donors with extended RBC antigen characterization is needed to support patients with sickle cell disease (SCD). Although identification of these donor units has historically been done serologically, automated DNA-based antigen testing can potentially improve the efficiency, reliability, and extent of matching for this heavily transfused population.

Challenges in Transfusion Management of SCD, Thalassemia, and Those With Warm Autoantibodies

Transfusion therapy is a key component of the comprehensive management of patients with SCD and thalassemia [3]. Current clinical guidelines recommend RBC transfusion for patients with SCD to treat acute complications including acute chest syndrome, stroke, aplastic crisis, and splenic sequestration, and before surgical procedures involving general anesthesia [4]. Primary and secondary stroke prevention is the major indication for chronic RBC transfusion therapy, with approximately 10% of children with SCD requiring regular transfusions [5]. Chronic transfusions are used to reduce ineffective erythropoiesis for patients with severe thalassemia (β thalassemia major) and are used intermittently for patients with milder forms of thalassemia [6].

Transfusion therapy for patients with SCD and thalassemia is complicated by high rates of alloimmunization. The prevalence of alloimmunization with ABO/Rh(D) matching alone ranges from 18% to 75% and from 4% to 37% in patients with SCD and thalassemia respectively [3,7]. The wide range of prevalence results from differences in exposure frequency, patient age (pediatric vs adult), and donor/recipient RBC antigen discordance. Alloantibodies to Rh (primarily C and E) and K comprise over two thirds of antibodies detected. In an effort to reduce RBC alloimmunization, many transfusion services have implemented prophylactic phenotype matching for C, E, and K antigens for patients with SCD and thalassemia. For SCD, this transfusion strategy has been associated with a reduction in alloimmunization prevalence from 18% to 75% (rate: 1.7 to 3.9 antibodies/100 U transfused) with ABO/Rh(D) matching only, to 5% to 24% (0.26 to 0.50 antibodies/100 U transfused) with C-, E-, and K-matched RBCs, and further reduced to 0% to 7% (≤0.10 antibodies/100 U transfused) with extended antigen matching (beyond C, E, and K) [8].

Despite receiving Rh phenotype–matched RBCs, many patients with SCD form Rh antibodies that may appear like autoantibodies since the patient's own RBCs type positive for the corresponding Rh antigen with standard serologic tests. RBC genotyping for *RHD* and *RHCE* has revealed that many of these patients carry variant alleles that encode partial D, C, and/or e antigens, and suggests that these were alloantibodies directed toward common Rh epitopes the patient lacks. Many of these Rh antibodies have been associated with laboratory evidence of delayed hemolytic transfusion reactions or demonstrated decreased survival of transfused RBCs [9,10]. Anti-D has also been reported in D+ patients with thalassemia, but it is unknown whether these patients had variant *RH* genotypes that could predispose them to D immunization [11].

Transfusion management of children with autoimmune hemolytic anemia or warm autoantibodies present additional challenges. In most cases, autoantibodies interfere with standard pretransfusion antibody screening and compatibility testing. This is most problematic if the child has been previously transfused and underlying alloantibodies have formed [12]. Time- and resource-consuming absorption techniques primarily available at reference laboratories are required for alloantibody evaluation and may not be effective in cases of high titer or tightly bound autoantibodies.

Table
Indications for RBC antigen genotyping

Clinical indication	Examples
Patient typing	
Recently or multitransfused	Hemoglobinopathies (SCD, thalassemia major/intermedia) Congenital or acquired hemolytic anemias
Presence of interfering antibodies	Autoantibodies, multiple alloantibodies, antibodies to high-prevalence antigens, antibody of undetermined specificity
Suspected antibody against antigens for which typing antisera are not readily available	$Do^{a/b}$, $Js^{a/b}$, $Kp^{a/b}$, V/VS RBC antigen variants
Serologic typing discrepancy	RhD typing discrepancy (due weak D, partial D)
To detect silencing genes or genes responsible of a weakly expressed antigen	To detect the GATA site mutation in the *DARC* gene in individuals who are Fy(b-)
Apparent autoantibody with antigenic specificity or unexplained antibodies despite antigen matching	Identify Rh variants (eg, anti-C, -e, or -D in patients typing C+, e+, or D+)
To identify fetal risk for HDFN	Paternal RhD zygosity testing Determining weak D status in pregnant females Prenatal fetal blood group antigen determination in allosensitized mothers
Blood donor typing	
To screen for antigen-negative and/or rare donors	Screening for rare combinations of antigen-negative RBCs Screening for *RH* genotype matched RBCs for SCD patients with anti-Rh antibodies[a]
To detect silencing genes	Screening for ethnic African blood donors by Fy(a-b-) phenotype[a]

[a] Large-scale donor molecular screening for *RH* variant phenotypes in African blood donors (established by Fy[a-b-] phenotype) has been reported to meet the transfusion needs of patients with SCD and anti-Rh antibodies [37].

Molecular Background of Blood Group Antigen Systems

More than 300 blood group antigens in 35 blood group systems exist, and the molecular basis for almost all blood group antigens and phenotypes is known. DNA-based prediction of a blood group antigen is simple and reliable for most antigens because most result from single nucleotide polymorphisms (SNPs). Molecular assay design and interpretation are thus straightforward for the prediction of most RBC antigen phenotypes, which are determined by a limited number of alleles or SNPs. However, DNA-based prediction of some blood group systems remains challenging, most notably the ABO and Rh systems due to the high number of variant alleles and their genetic complexity. In the ABO system, more than 100 alleles encode the glycosyltransferases responsible for the ABO type, and a single nucleotide change in an A or B allele can result in an inactive transferase and a group O phenotype. Mistyping of ABO antigens could lead to transfusion of ABO-incompatible blood and a subsequent severe hemolytic transfusion reaction. Genotyping methods have been developed to decrease the risk for erroneous ABO prediction [13] but are unlikely to replace ABO typing by hemagglutination, which is extremely reliable, inexpensive, and has a quick turnaround time.

In the Rh system, molecular testing for the common Rh antigens D, C, c, E, and e is uncomplicated for most populations. The *RHD* and *RHCE* genes are each composed of 10 exons in opposite orientation and share 97% nucleotide sequence homology. Their close proximity, sequence homology, and opposite orientation have resulted in many variant and hybrid alleles evolving on both loci [14]. Over 200 *RHD* alleles encoding partial and weak D, and approximately 100 *RHCE* alleles encoding altered or partial Rh antigens have been described.