

Integrating Molecular Technologies for Red Blood Cell Typing and Compatibility Testing Into Blood Centers and Transfusion Services

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Nucleic acid–based technology is now at a point where the field of transfusion medicine is ready for its widespread application. In the donor center, genotyping of red blood cell (RBC) products provides phenotype-matched products for special patient populations or antigen-negative products for patients with alloantibodies. In the immunohematology reference laboratory, molecular technologies aid in discerning blood types in the situation of a typing discrepancy and improve pretransfusion RBC testing reagents. In the hospital transfusion service, genotyping patients aids in providing phenotype-matched RBC products. In prenatal testing, genotyping for *RHD* aids in the decision for Rh immune globulin prophylaxis and predicting risk of

hemolytic disease of the fetus and newborn. Before genotyping is accepted as the universal standard for pretransfusion and donor testing, important limitations of this technology must be addressed, including the fact that the genotype does not always predict the phenotype and the need for creating the ideal high-throughput platform. Clinical trials are needed to answer important questions, and a donor and patient database is needed. A stepwise plan for progressive introduction into the donor centers and transfusion services must be established. In conclusion, the field of transfusion medicine is ready to expand the use of molecular diagnostics.

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FOR OVER A century, hemagglutination has been the gold standard for red blood cell (RBC) antigen detection in the determination of a patient's RBC phenotype. Hemagglutination implies that RBC antigen and antibody reagents interact, and this interaction has allowed the terms "serology" and "immunohematology" to become common parlance. However, despite its relatively low cost, ease of performance, and sensitivity and specificity suitable for what we now consider to be optimal patient care, hemagglutination-based determination of RBC phenotype has its limitations, which are described below. In part, to mitigate these limitations, nucleic acid–based technologies have been added to the armamentarium of methods available in advanced immunohematology reference laboratories, thus allowing the term "molecular immunohematology" to enter the blood banking lexicon. Molecular immunohematology refers to the use of genotyping applied to the genes encoding RBC antigens. Thus, this technology represents an indirect method for predicting an individual's RBC blood group phenotype. Initially assumed to be complex, costly, and difficult to automate, it appeared that RBC blood group antigen genotyping would not be ready, in the reasonably foreseeable future, for routine application. However, advances in our understanding of the relationship of RBC genotype to phenotype, detailed description of the sequence and function of blood group genes and related silencing elements, concepts of nano and chip technology applied to RBC nucleotide polymorph-

ism detection, and the concept that genotyping might be a valuable complement to hemagglutination, it is likely that genotyping technologies will see widespread adoption in both the blood center and the hospital transfusion service. This approach will primarily be used initially in specialized clinical situations and in immunohematology reference laboratories servicing more complex patients. Thus, transfusion authorities are now starting to consider the use of molecular techniques in transfusion medicine as critical to advancing the understanding of blood group antigen polymorphisms and to increasing blood safety by providing better-matched, compatible homologous blood products for transfusion. The purpose of this review is to present the current status of molecular immunohematology as applied to donor centers, reference laboratories, and transfusion services. We also consider what clinical trials and regulatory aspects might be needed and the apparent path to a stepwise implementation and adoption plan for this important and emerging technology.

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RED BLOOD CELL ANTIGENS AND THEIR GENES AND GENOTYPING

There are approximately 270 serologically determined RBC antigens. Initial recognition of the allelic nature of serologically defined antigen differences led to the concept that single nucleotide polymorphisms (SNPs), which lead to single amino acid differences in RBC antigens, could be used to precisely determine an individual's phenotype via clear relationships between RBC antigen genotype and phenotype. Well-known examples of this concept are Gly42Asp being the amino acid change associated with Fy^a/Fy^b determinants, Thr193Met for K/k, and Met29Thr for S/s. However, detailed serologic and molecular/genetic investigations over many years also confirmed that some RBC antigen genes and their corresponding protein or carbohydrate moiety antigens are not as simple as formerly thought. There can be multiple genetic variations to account for the same blood group phenotype. For example, there are over a 100 alleles for the 4 ABO phenotypes.¹ This tremendous complexity of alleles encoding RBC antigens, now confirmed with genotyping, and the corresponding antibody formation, is increasingly compounded by globalization of populations and heterogeneous RBC exposure through transfusion. Another example of this complexity is demonstrated by the current understanding of the MNS blood group system, which is highly polymorphic and includes at least 43 antigens. Many of the antigens are uncommon, resulting from an amino acid substitution or a

Table 1. Limitations of Serologic Immunohematology

Technical limitations
Subjective interpretation
Labor intensive procedure requiring manual data entry
Requires use of reliable antisera
Cost of FDA-approved reagents is escalating
Many antisera used are not FDA approved
Antisera are often limited in volume, weakly reactive, or unavailable
Source material is a biohazard serving as a potential reservoir of infectious disease
Clinical limitations
Typing of RBCs from patients who have been recently transfused
Typing of RBCs from patients with autoantibodies
Does not precisely determine <i>RHD</i> zygosity in D-positive individuals
Small number of donors are typed for a small number of antigens, limiting antigen-negative or rare donor registries

Table 2. Molecular Events That Give Rise to Blood Group Antigens and Phenotypes³

Single-nucleotide substitution in coding sequences and intronic sequences involved in splicing (most blood group systems)
Deletion of a gene, exon, or nucleotide(s) (ABO, MNS, Rh, Kell, Duffy, Dombrock, Gerbich, etc)
Gene conversion or recombination events, which have been recognized for several blood groups, especially those encoded by clustered gene loci (namely, MNS, Rh and Ch/Rg)
Insertion of a nucleotide(s) (Rh, Colton)
Duplication of an exon (Gerbich)

rearrangement between *GYP A* and *GYP B*. Low-prevalence antigens in the MNS blood group system include Cl^a, DANE, Dantu, ERIK, Far, HAG, He, Hil, Hop, Hut, MARS, M^c, M^g, Mi^a, MINY, Mit, Mt^a, Mur, MUT, M^v, Nob, Ny^a, Or, Os^a, Ri^a, s^D, SAT, St^a, TSEN, Vr, and Vw. There are rare null phenotypes in this system that result from gene deletions, namely, En(a-), which lacks MN antigens; U-, which lacks Ss antigens; and M^kM^k, which lacks both MN and Ss antigens. Finally, some antigens that are associated with the MNS system but are not numbered by the International Society of Blood Transfusion (ISBT) Working Party on Terminology for Red Cell Surface Antigens are a consequence of altered glycosylation at residues 2, 3, and 4 of glycoporphin A (GPA). These include Tm, Sj, M₁, Can, Sext, and Hu. Thus, the genetic, protein and carbohydrate complexity of antigenic differences has made serologic determination of the wide range of RBC antigens quite challenging. These challenges can be differentiated into Technical Limitations and Clinical Limitations as summarized in Table 1.

To date, genes encoding 28 of the 29 established blood group systems have been cloned and sequenced; only the encoding of the P system remains to be resolved.² The variety of different molecular events that can occur in the generation of blood group antigens is summarized in Table 2.³ As above, most of these occur because of a SNP, which can be exploited in the use of genotyping.

During the past 10 years, experimental immunohematology laboratories have implemented molecular methods to identify specific SNPs in the many genes that encode blood group antigens. In addition, there has been the development of several different mass-scale genotyping technologies to perform high-throughput blood group prediction with the goal of mainstream application.

Table 3. Application and Implementation of Molecular Technologies

Donor center
<i>Genotype RBC products</i>
Product for special patient populations, such as sickle cell disease patients
Products for patients with multiple alloantibodies
<i>RHD</i> genotyping donors who are D-negative
<i>Reference laboratory</i>
Reagent RBCs for antibody detection
Genotype to determine dosage of RBC antigens
Resolution of typing discrepancies
Genotype to predict presence or absence of an antigen when no antisera exists
Determination if new antibody is an autoantibody or alloantibody
Resolution of unusual serological findings
Transfusion service
<i>Genotype patients</i>
Recently transfused patients
Patients with autoantibodies
D type of the patient to predict need for Rhlg or D-negative products
<i>Providing genotyped matched products</i>
Patients with SCD
Patients with thalassemia
Patients with AIHA
Chronically transfused patients
<i>Prenatal testing</i>
<i>RHD</i> type to predict need for Rhlg
Genotype fetal DNA to predict risk for HDFN

Abbreviations: SCD, sickle cell disease; AIHA, autoimmune hemolytic anemia; HDFN, hemolytic disease of the fetus and newborn.

The methods use amplification of the target gene sequence by polymerase chain reaction (PCR) followed by analysis using restriction fragment length polymorphisms, real-time PCR, sequence-specific primer PCR either single or multiples, single base extension, high-throughput bead technology, or microarrays. These technologies are recently reviewed in an article by Avent.⁴ The application and implementation of molecular technologies in the blood center, reference laboratory, and transfusion service is presented in Table 3.

DONOR CENTER

Phenotyping RBC Products

In the United States, the current practice is to only match for the ABO and D antigens, but there are 2 situations when multiple antigen-negative RBC products are requested: (a) when recipients have corresponding alloantibodies and (b) for special

chronically transfused patient populations, such as patients with sickle cell disease (SCD).

Alloimmunization occurs in approximately 2% to 6% of patients who receive RBC transfusions, but the rate of alloimmunization may be as high as 36% in patients with SCD.² Recipients with multiple alloantibodies impair the ability to provide antigen-negative, compatible RBCs for transfusion because blood banks must phenotype many times the number necessary to find an appropriate product. The numbers of products screened multiplies depending on the RBC antigen prevalence and the number of negative antigens necessary, thus placing a burden on the donor center needing to identify such products. The burdens include laboratory technologist time, adequate RBC inventory, and appropriate reagents. A major goal of the mass-scale genotyping process is to allow for the expansion of phenotype/genotype matching for a greater number of patients, thereby improving patient care.

There are patient populations that benefit from receiving phenotype-matched products, especially those who are chronically transfused or at increased risk for alloantibody formation. Phenotype-matched products can be limited to the C, E, and K antigens or extended to include Fy^a, Jk^a, Jk^b, S, and other antigens. The fundamental reason to phenotype-match products is to prevent alloantibody formation and the subsequent negative consequences of hemolytic transfusion reactions. The downside of this precise matching practice is that it makes routine transfusion more difficult for both the donor center and transfusion service. Therefore, currently phenotype-matching is only applied to specific patient populations.

Donor centers currently screen and stock RBC products to keep a pool of frequently needed antigen-negative products. Usually, donor centers screen products from repeat group O donors and family members of patients who have formed alloantibodies to high-prevalence antigens for rare blood types. Batch serologic screening is technologist time-intensive to perform; typing reagents are expensive; and appropriate controls are required. As a result, donor centers have algorithms to help determine the likelihood of an antigen-negative product based on limited phenotyping and donor race and ethnicity.

The American Rare Donor Program (ARDP) is a list of over 30 000 individuals compiled from AABB and the American Red Cross who are active blood

donors with a blood type that occurs in less than 1 in 10 000 people.⁵ This program supplies these rare RBCs all over the world, but mostly within the United States. It relies on the above methods to find these products and the continued good will of these donors to maintain an adequate supply. In addition, the ISBT maintains a rare blood donor program, which is compiled and maintained by the International Blood Group Reference Laboratory.⁶ The use of large-scale genotyping methods would enable increased identification of these RBC products and corresponding donors. Hashmi et al⁷ demonstrated the above concept when they genotyped 2355 donors using the BeadChip to predict for the presence of K, k, Jk^a, Jk^b, Fy^a, Fy^b, M, N, S, s, Lu^a, Lu^b, Di^a, Di^b, Co^a, Co^b, Do^a, Do^b, Jo^a, Hy, LW^a, LW^b, Sc¹, Sc², and HgbS. They were able to identify 21 rare donors (Co(a-b+), Jo(a-), S-s-, and K+k-).

Red Blood Cell Product Genotyping

High-throughput molecular technologies in the field of transfusion medicine make it possible to determine multiple RBC antigen SNPs simultaneously. There are multiple mass-scale genotyping platforms in use and in development in North America and Europe.

Since 2000, the BloodGen project has aimed to develop and standardize mass-scale molecular genotyping using a microarray platform known as the Bloodchip manufactured by Progenika Biopharma (Vizaya, Spain).⁴ This platform includes genotypes for ABO, *RHD*, *RHCE*, *KEL*, *FY*, *JK*, *DI*, *CO*, *MNS*, and *DO* blood groups and selected human platelet antigen alleles. The BloodGen project has performed multiple small-scale clinical trials, initially using samples from individuals with rare blood types. This exercise has been repeated to optimize the design of the Bloodchip, which is a tactic used by other high-throughput platforms as well. Currently, there is a large clinical trial with the goal of bringing this product to market.

GenomeLab SNPstream by Beckmann Coulter (Fullerton, CA) and BeadChip developed by BioArray Solutions (Warren, NJ) are also being developed for mass-scale genotyping of RBC donors.⁴ Recently, the human erythrocyte antigen BeadChip array was used to determine 24 antigens within 10 blood group systems for 2355 American RBC donors.⁷ The donors represented a diverse population of whites, African Americans,

Hispanics, and Asians. There was nearly complete concordance of genotypes and serologic phenotypes; however, of the discordant results, confirmatory testing including gene sequencing and restriction fragment length polymorphism analysis favored the molecular method (n = 16) or was resolved by manual DNA analysis of *GYPB* exon 5 mutations (n = 8). These clinical trials confirmed the importance of including relevant silencing SNPs.

Mass-scale genotyping of RBC donors would enable increased provision of RBC antigen-matched products to recipients, especially those special populations who are at an increased risk for alloimmunization. In addition, mass-scale genotyping would expand the genotyped/phenotyped donor database and increase the number of rare donor products, which would improve patient care.

D Typing

The donor center must ensure that D-negative products are appropriately labeled, such that a recipient of a D-negative product does not form anti-D in response to transfused RBCs. Currently, donor centers use typing reagents to detect the presence of the D antigen, and to increase the sensitivity of these reagents, the test is brought to the antiglobulin phase (weak D testing), which takes additional time, reagents, and controls.⁸ If the donor's RBCs have a positive direct antiglobulin test, the antiglobulin phase will be falsely positive, and additional work must be done to determine the D type. Wagner et al⁹ screened 8442 D-negative blood donations by *RHD* sequence-specific primer PCR and detected 5 D-positive donors. One donor was a D-positive, D-negative chimera with 94% D-negative RBCs. They traced 13 previously donated products to 2 D-negative recipients who had formed an anti-D alloantibody after transfusion. In addition, they found 45 D-negative yet *RHD* gene-positive (albeit silenced) samples, which mostly represented novel *RHD* genes.⁹ Approximately, 30 000 D-negative donors in Germany have been *RHD* tested, and about 1 in 1000 express the D antigen and are subsequently removed from the D-negative donor pool.¹⁰ In a recent study from Austria, 3 of 2427 D-negative donors carried *RHD* (1 weak D and 2 D_{el}), which is an estimated incidence of 0.12%.¹¹ In conclusion, the use of molecular typing will prevent mislabeling of 0.1% of donors

as D-negative and avoid potential alloimmunization in the recipient.

REFERENCE LABORATORY TESTING

Reagent RBCs in Antibody Detection

The detection and identification of RBC alloantibodies rely on the use of well-characterized reagent RBCs. Lack of hemagglutination between the patient's plasma and RBCs ideally from a person homozygous for the genes expressing the presence of the antigen is used to exclude the corresponding alloantibody. Currently, serologic methods are used for phenotyping RBC reagents used for antibody screening and the identification of panel RBCs. There are blood groups where the determination of homozygosity is notoriously difficult, especially Fy^a/Fy^b and D. In addition, there are blood group antigens where serologic testing cannot be performed; this is particularly true for low-prevalence antigens. When low-prevalence RBC antigens are present on screening cells but are not identified, they may lead to a positive antibody screen, and further workup reveals no identifiable alloantibody. Thus, the patient's plasma must be crossmatched to RBC products often without the knowledge of the alloantibody's identity. From a logistic and patient care perspective, the appropriate antigen-negative product cannot be preselected in the future, especially when the reactivity disappears from the patient's plasma or the screening RBCs lack the antigen. In addition, additional time and resources are often used without benefiting and, in fact, delaying patient care. At the Blood Center in Sweden, 3 of 52 reagent RBCs, which had been predicted by phenotype to be from an *RHD* homozygote, were in fact from a hemizygote by genotype and thus expressed a single dose of D.¹² Of the 74 Fy^a or Fy^b RBC samples predicted by phenotype to be from a homozygote person, 7 were found to be hemizygous by genotype due to the presence of either the *FY*X* or the *FY*O* genes.¹³ In addition, reagent RBCs were genotyped for *DO*. Antibodies to Do^a and Do^b have resulted in hemolytic transfusion reactions, but the antibodies are difficult to identify due to lack of adequate typing reagents and, therefore, identification of the antigen on various RBC reagents used. Genotyping DNA from donors of reagent RBCs allows for improved prediction over serology that the RBCs carry a double-dose expression of the antigen for use

to exclude the presence of RBC alloantibodies, possibly reducing the finding of "nonspecific" reactivity, and for predicting reagent RBC antigen phenotype when no typing reagents are available.

Resolution of Typing Discrepancies

Red blood cell genotyping can be used to resolve weak hemagglutination reactions and typing discrepancies, most often encountered as a result of variation in the ABO and D phenotypes. The ability to accurately determine an individual's antigen status would eliminate the use of antigen-negative blood where the patient would be unlikely to become alloimmunized, the use of group O RBCs and AB plasma for transfusion in the situation of ABO typing discrepancy, or the loss of a product in the donor setting because of the inability to appropriately label it. Olsson et al¹⁴ investigated 324 RBC samples with ABO typing discrepancies. They investigated samples with acquired variant ABO phenotypes from pregnancy, hematologic disorders, and other medical conditions, samples with inherited ABO phenotypes due to known and unknown subgroup alleles, samples of relatives of these subgroup individuals, samples with unexpected absence of or weakly reactive anti-A or anti-B, and samples of suspected chimerism. Thus, genotyping can aid in the differentiation between subgroup alleles and acquired weakened agglutination and allows proper ABO identification of both donors and patients.

Providing Antigen-Negative Products When No Typing Reagents Exist

Monoclonal antibodies prepared from human/mouse heterohybridoma cell lines or polyclonal antibodies from human plasma are used as typing reagents. These reagents usually meet Food and Drug Administration (FDA) potency requirements, but some RBC typing reagents are either not available or made from saved patient's plasma with the desired antibody specificity. These human-derived antibodies may be only weakly reactive, formed in conjunction with other antibodies or in individuals with anti-A and/or anti-B present, making their use difficult for some RBC samples. Antibodies, including anti-A and/or anti-B can be removed through absorption, but this is a time-consuming process and may dilute the wanted antibody's strength. These typing reagents can be difficult to obtain. If antisera are not available, then

a crossmatch with the patient's plasma to detect the presence of the RBC antigen must be relied on. If the patient's antibody titer is too low to detect, then there is no method to ensure that the products are antigen-negative and the patient may receive antigen-positive products. Reid¹⁵ described the use of genotyping to find needed RBC products when antiserum was not available for the Dombrock antigens. DNA analysis allowed for the successful transfusion of Do^a and Do^b antigen-negative products. The concept of genotyping rather than relying on rare antisera or patient crossmatch results, if the antibody still persists, is vital to the prevention of possible hemolytic transfusion reactions in such patients.

Alloantibody Versus Autoantibody

When patients have been transfused and no pretransfusion specimen is available, then the determination of a newly identified antibody is against foreign cells (allo) or self cells (auto) can be difficult. An example is a patient whose RBCs type as D-positive and forms an anti-D after transfusion of D-positive products. The only way to determine if the antibody is to the transfused RBCs is to separate the transfused from the patient's RBCs and determine to which the antibody binds. Using molecular methods, the patient's *RHD* genotype would predict if their phenotype was one at risk at making anti-D. Such phenotypes are known as partial D. In the case of an autoantibody, transfusion of antigen-positive products is possible, but in the case of an alloantibody, it could lead to a hemolytic transfusion reaction and should be discouraged.

TRANSFUSION SERVICE

Genotyping the Patient

Recently transfused patient. Patients who are transfusion dependent, such as those with SCD, thalassemia, and aplastic anemia, are difficult to phenotype when no pretransfusion sample is available. Because of the presence of circulating donor RBCs, which may persist for weeks, determination of an individual's phenotype by traditional hemagglutination methods is complex due to the presence of a mixed field population. With the use of time-consuming and labor-intensive techniques, such as isolating reticulocytes or sickle cells (in individuals with SCD), the RBC phenotype may be determined, but it may be inaccurate.

Molecular methods can overcome this limitation of hemagglutination. Because transfusion has no effect on somatic cells, the use of buccal epithelial cells and urine sediment as a source of DNA can provide accurate results. It is also possible to obtain DNA from white blood cells present in the peripheral blood; however, some have criticized that interference from circulating donor white blood cells may have an effect on the result. However, most PCR assays used in transfusion medicine do not detect posttransfusion DNA chimerism.¹⁶⁻¹⁸ Rozman et al¹⁶ demonstrated identical RBC typing on pretransfusion, posttransfusion, and buccal samples in 8 patients after 26 multiple-transfusion events. In addition, no differences were identified between the numbers of RBC products transfused including those that were not leuko-reduced or the time elapsed between transfusions and testing. In summary, the determination of a recently transfused patient's RBC genotype is superior to using traditional hemagglutination techniques.

The patient with a positive direct antiglobulin test. Patients with circulating autoantibodies, with or without autoimmune hemolytic anemia (AIHA), can complicate RBC phenotyping. In patients with warm autoantibodies, the use of indirect antiglobulin reactive reagents may result in false-positive antigen typings unless the IgG can be removed from the patient's RBCs before testing. Fortunately, many RBC typing reagents now consist of monoclonal antibodies allowing for antigen detection in the direct agglutination phase.¹⁹ There are situations when no direct agglutinating reagents are available, when the antigen is sensitive to the IgG removal treatment, or when the IgG removal is not effective.¹ In such cases, genotyping provides an acceptable alternative by allowing the prediction of a patient's RBC antigen status without the interference of the autoantibody.

D status of the patient. Current D-typing reagents in use in both donor centers and transfusion services may have difficulty in determining the status of weak D or partial D individuals. Individuals with partial D, whether strong, variable, or weak expression of D, are at risk for forming an anti-D. These patients would benefit from receiving D-negative RBCs for transfusion and potentially benefit from Rh immune globulin (RhIg) prophylaxis. Patient typing is usually performed with an IgM monoclonal anti-D reagent that does not detect, in the direct phase, DVI, which is the most

Table 4. Weak D Types That Are Prevalent or Clinically Relevant¹⁰

Weak D phenotype	Prevalence in Germany (%)	Haplotype association	Recommended management	
			RBC product transfusion	Rhlg in pregnancy?
Type 1	0.2964	<i>CDe</i>	D-positive	No
Type 2	0.0759	<i>cDE</i>	D-positive	No
Type 3	0.0219	<i>CDe</i>	D-positive	No
Type 4.0	0.0140	<i>cDE</i>	D-positive	No
Type 4.1	0.0230	<i>cDe</i>	D-positive	No
Type 4.2	Rare	<i>cDe</i>	D-negative	Yes
Type 5	0.0035	<i>cDE</i>	D-negative	Yes
Type 11	>0.0009	<i>CDe</i>	D-negative	Yes
	0.0009	<i>cDe</i>		
Type 15	Rare	<i>cDE</i>	D-negative	Yes
Type 19	0.0670	<i>CDe</i>	D-negative	Yes
Type 20	0.0240	<i>cDE</i>	D-negative	Yes
Other types	Rare	<i>Variable</i>	D-negative	Yes

common form of partial D in whites.²⁰ The practice of Flegel¹⁰ is to genotype all patients with serologic reactivity of 2+ or less in gel (about 1% of patients) to resolve their weak D type at the molecular level. Based on their molecular type, the patient is transfused with D-positive or D-negative RBCs as appropriate and is evaluated for the need for Rhlg during pregnancy (Table 4).¹⁰ This logical combination of the use of phenotyping followed by genotyping leads to improved patient care and presumed less D alloimmunization. Unfortunately, at this time, there are no guidelines about the need to determine the weak D type or the transfusion management of these patients. The increased use and data collection of molecular weak D types would create a better understanding of how to best manage these patients.

Providing Phenotyped-Matched RBCs

Sickle cell disease. SCD is the most prevalent genetic disorder in the African American population.²¹ These individuals are usually homozygous for the hemoglobin S gene, but such patients can also carry HgbS along with β thal⁰, β thal⁺, and HgbC. SCD affects approximately 80 000 people in the United States with a prevalence of 1 in 400 in African Americans and a carrier rate of 1 in 12.²² Patients with SCD live on average 45 years secondary to improved management, which includes the use of RBC transfusion.²³ In a study looking at adult and pediatric patients with SCD, 46% of the children were transfused a mean of 24 products, whereas 87% of the adults were transfused a mean of 24 products over a 10-year

period.²⁴ High alloimmunization rates are postulated to result from the antigenic disparity between African Americans and whites, which is most strikingly seen in transfused patients with SCD. Alloimmunization often makes finding compatible RBC products difficult and increases the risk of delayed hemolytic transfusion reactions (DHTRs). Without the implementation of extended phenotype matching in patients with SCD, studies reported an alloimmunization rate in the range of 19% to 43% in transfused patients with SCD.²⁵ One study reported an alloimmunization rate of 29% in pediatric and 47% in adult patients with SCD, with more females than males being immunized. The number of delayed hemolytic and/or serologic transfusion reactions was 8% (adult) and 9% (pediatric), and the incidence of hyperhemolysis was 1.6% (adult) and 5.1% (pediatric).²⁴ In contrast, multiple-transfused non-SCD, nonblack, chronic anemia patients with either thalassemia major or pure red cell aplasia had an alloimmunization rate of approximately 5%.²⁵ In a comprehensive study of the incidence of, and risk factors associated with, alloimmunization in patients with SCD, Vichinsky et al²⁵ suggested that the increased alloimmunization rate in patients with SCD was likely due to antigenic differences between patients with SCD (most of whom were of African descent) and most blood donors (most of whom were white).^{26,27} The most common alloantibodies found are against the K, E, C, and Jk^b antigens, which are related to the antigenic prevalence in donors versus patients with SCD (respectively, K 9% vs 2%, E 35% vs 24%, C

68% vs 28%, and Jk^b 72% vs 39% are positive).²⁵ Therefore, phenotype-matched RBCs for the high likelihood antigens (Rh and K) result in decreased alloimmunization.

In addition to RBC antigen mismatch, a recipient's inflammatory status may play a role in the risk of alloimmunization.²⁸ This concept has been hypothesized for humans and demonstrated in mice by two separate studies.^{29,30} In patients with SCD, absolute steady-state neutrophil count has been correlated with clinical severity.³¹ As a correlate to this, patients with SCD who respond well to hydroxyurea have a decrease in their neutrophil count. Thus, as inflammation appears to be associated with sickle exacerbation, which is treated with transfusion, patients with SCD may be selectively exposed to RBC alloantigens when in an inflamed state. However, it is currently unknown if patients with SCD with higher neutrophil counts are at increased risk of alloimmunization.

Providing partially phenotype-matched RBC products for the treatment of patients with SCD has been advocated to minimize the likelihood of RBC alloimmunization.³² One such study showed a decrease in the alloimmunization rate from 35% to 0%, with the exclusive use of phenotype-matched RBCs (C, c, E, e, K, S, Fy^a, and Fy^b) in a 12.5-year period.³³ Afenyi-Annan and Bandarenko³² surveyed hospitals in North Carolina about SCD transfusion practices and demonstrated that only 17% of prophylactically provided antigen-matched products, which is in contrast to most major academic medical centers. Among the academic centers, the protocol for antigen-matching was inconsistent as to which antigens were matched; 73% matched for E, 70% for K, 68% for C, 41% for c, and 41% for e. Therefore, providing phenotype-matched RBC products for SCD is inconsistently practiced yet important to the care of these patients.

One study has applied genotyping to chronically transfused patients with SCD. Castilho et al³⁴ found discrepancies in 6 of 40 patients between the serologic RBC phenotype and phenotype predicted by the genotype in the patients with SCD but not the control group. The serologic phenotype mistypes were secondary to recent transfusion. The 6 patients who then were switched to the correct antigen-matched RBCs had improved RBC survival with diminished frequency of transfusions. This study highlights the use of genotyping patients with SCD

for antigen-matched RBC transfusions and the resulting improved patient care.

β-Thalassemia. Thalassemia is a hereditary anemia resulting from defects in the β-globin chain, which can lead to a chronic severe anemia.³⁵ The disease is clinically heterogeneous due to genotypically different mutations or compound heterozygosity with other hemoglobinopathies and to unknown individual patient factors. Patients with thalassemia may require life-long RBC transfusions to ameliorate the chronic anemia and to suppress the extramedullary hematopoiesis, which would otherwise lead to severe bone deformities. RBC alloimmunization occurs at a rate of 5% to 33% depending on the homogeneity of the population.^{36,37} Alloimmunization rates were lowered by phenotype-matching for Rh and Kell from 33% to 2.8% at Children's Hospital Oakland and 23.5% to 3.7% at Aghia Sophia Children's Hospital.³⁶ In addition, the treatment of these patients can be complicated by the presence of RBC autoantibodies. Because these patients are transfused from birth, RBC phenotyping should be performed on the initial pretransfusion sample or genotyping must be used to adequately predict the patient's RBC antigen type. Castilho et al³⁸ genotyped 10 alloimmunized β-thalassemia patients for E, e, K, k, Fy^a, Fy^b, Jk^a, and Jk^b who had been receiving antigen-matched RBC transfusions based on phenotype. In 9 of 10 patients, there was a discrepancy between the historical phenotype and the phenotype predicted by the genotype, 5 in the Rh system, 3 in the Kidd system, and 1 in the Duffy system. The discovery of these discrepancies aided in the identification of alloantibodies and the selection of the correct antigen-matched products.³⁸ Genotyping of RBC transfusion-dependant patients is vital to preventing and identifying alloimmunization and providing appropriate antigen-matched products.

Autoimmune hemolytic anemia. During an acute presentation in a patient with newly diagnosed AIHA, finding the appropriate RBC product for transfusion can be a challenge, and close communication between the transfusion service and the treating physician is necessary. Because of the presence of a strong autoantibody, the antibody screen and identification panels will show panagglutination, making it difficult to detect or exclude underlying RBC alloantibodies. Absorption techniques using either donor or patient RBCs are

available at some hospital laboratories, but many must send samples to reference laboratories for these specialized tests, which are time consuming. Of patients with warm autoantibodies, 20% to 40% have clinically significant alloantibodies.³⁹ Performing the RBC phenotype in these patients is helpful because it focuses the antibody workup on the possible alloantibodies the patient is capable of forming. In addition, if a complete phenotype can be determined, then the transfusion service can provide antigen-matched RBCs, which may prevent future alloimmunization and DHTRs as well as circumvent the absorption studies. The John Hopkins Hospital published their approach to patients with warm autoantibodies, which included a phenotype for C, E, c, e, K, Jk^a, Jk^b, Fy^a, Fy^b, S, and s, if they were able to perform the phenotype and providing antigen-matched as well as antigen-negative for any identified alloantibodies.³⁹ Phenotyping may not be possible if the IgG dissociation method is unsuccessful, when only antiglobulin-reactive antisera is available, if the antigen is denatured by the dissociation method, or if the patient has been recently transfused. During analysis of subsequent samples, if the serologic findings were consistent with previous findings, then phenotype-matched products were provided. Of the 20 patients, 12 could be fully phenotyped and 8 patients could be partially phenotyped or phenotyping was indeterminate. The patients received between 2 and 39 products, and none developed new alloantibodies during the study period of 13 months. Garratty and Petz,⁴⁰ in an accompanying editorial, reported 202 alloantibodies to 37 different antigens detected in the sera of 418 patients with AIHA; 15% of these alloantibodies would not be covered in the Hopkins' protocol, but most of these uncovered antibodies were to low-prevalence antigens and unlikely to cause hemolysis. A second potential criticism of this protocol is that the prevalence of phenotypes in these patients ranged from 0.0002 to 0.09, which may make it highly unlikely to find a matched RBC product. If genotyping were available, then prophylactic antigen-matching without future absorption studies could have been performed on all the patients, simplifying future laboratory pretransfusion testing and improving delivery of appropriate RBCs for transfusion in a more timely manner.

All patients. A pilot study from the Hong Kong Red Cross demonstrated the feasibility of pheno-

typing all citizens, placing the results on a smart card and providing phenotyped-matched products (for ABO, C, c, E, e, D, K, k, M, N, Fy^a, Fy^b, Jk^a, and Jk^b) without the need for pretransfusion testing.⁴¹ These investigators were able to provide 395 RBC products for 92 patients almost entirely from the hospital, which stocks 300 products, and rarely needed products supplied from the Hong Kong Red Cross donor center, which stocks 4000 products. For recipients with uncommon phenotypes, only 0.2%, the probability of finding a phenotype-matched product was 0.451 in the hospital inventory and 0.999 in the Red Cross inventory. The cost of phenotyping was offset by eliminating pretransfusion testing. The benefits of a phenotype-matched system over the current antibody screening system are the time saving of not having to collect blood samples or test them and the decrease of mismatched transfusion secondary to the ability to electronically match the patient data and the RBC product data. In conclusion, this practice is feasible and cost-effective if an adequate inventory is maintained in the hospital and donor center, which is adequately representative of the patient population, as well as reliable communication between the donor center and transfusion service.

Prenatal Testing

RhIg use. Current D typing reagents have difficulty in determining weak D or partial D individuals. Individuals with partial D, and some with weak D, are at risk for forming anti-D. These patients would benefit from receiving RhIg prophylaxis. Molecular testing of *RHD*, especially in patients with weak expression of D, would aid in the determination if RhIg is necessary (Table 3).¹⁰ Domen⁴² surveyed more than 3000 hospitals in the United States about their practices to test for weak D and administration of RhIg; 58% perform weak D testing and 31.8% had at least 1 patient with the weak D phenotype who formed an anti-D alloantibody. Testing for weak D in pregnant women is optional; the usual practice in the United States is to perform weak D test and not give RhIg to patients who are clearly D-positive or weak D-positive.⁴³ This prevents women with weak D antigens who do not need RhIg from receiving it but may lead to women with partial D antigens, such as those with D^{VI}, who theoretically need RhIg from not receiving it. Another argument used for not treating

weak D women as RhIg candidates is that weak D expression will cause a false-positive rosette test for fetal maternal hemorrhage, but sending the sample for Kleihauer-Betke acid elution method can circumvent this. The addition of molecular testing for weak D patients would aid in the decision of the use of RhIg prophylaxis.

Hemolytic disease of the fetus and newborn.

The management of a newly identified clinically significant alloantibody in a pregnant woman relies on serologic testing as well as phenotyping and rarely genotyping. If paternity is assured, the father is phenotyped to assess for risk of hemolytic disease of the fetus and newborn (HDFN). If the father's RBCs do not carry the antigen, then no further workup needs to be performed. If the father is homozygous for the gene expressing the antigen, the fetus is at risk. If the father is heterozygous for the gene expressing the antigen, then the fetus has a 50% chance of being at risk. Amniocentesis provides samples for fetal genotype (if needed), amniotic fluid spectral analysis, and fetal lung maturity. Alternatively, fetal DNA can be extracted from the maternal plasma to help determine antigen status of the fetus, but currently, this is not routinely available in the United States.⁴⁴

PRACTICAL CONSIDERATIONS

Limitations

Most of the criticisms surrounding RBC antigen prediction by molecular methods have been the cost and the uncertainty of regulations needed to implement this technology. However, one of the biggest limitations to consider is that genotyping may not accurately predict the antigen type. In the donor setting, false-positive results for the prediction of the presence of a RBC antigen would eliminate the donor as being suitable for a patient requiring an antigen-negative product, but it would not jeopardize the potential transfusion recipient. For example, a product falsely labeled Fy(b+) would have no negative consequences for the recipient. However, false-negative results could potentially lead to a hemolytic transfusion reaction. Therefore, a system to confirm the absence of antigens is necessary. Confirmation by hemagglutination methods, when suitable reagents are available, and/or a full crossmatch is recommended.

In addition, medical procedures such as stem cell or bone marrow transplantation, certain solid organ transplants, and natural chimerism may lead to mixed DNA populations. In patients with a history of transplantation, their genotyping results may change over time in parallel with their chimerism status; this would be influenced by which cells were used as the source of DNA. Therefore, an accurate patient history should be obtained, and in transplantation patients, repeat samples may be necessary.

Another limitation is that not all blood group antigens are a consequence of SNPs, which results in the need for more sophisticated testing methodologies or testing algorithms to use the genotype to predict the phenotype. For example, silencing mutations can be detected in a gene, but the gene may not be expressed, and therefore, the individual will phenotype as being negative for the antigen.

Cost

With the development of high-throughput systems, the cost of genotyping has dramatically decreased. Costs surrounding the BloodGen project have been estimated to be one Euro per SNP with at least 116 SNPs being analyzed. With mass-scale application, the cost may decrease further.⁴

Direct cost comparisons between genotyping and phenotyping are difficult and will vary depending on the number of antigens and which antigens to genotype versus phenotype. If no antisera are available for the corresponding antigen, then genotyping should be performed. ABO is straightforward and inexpensive to phenotype yet relatively complicated to genotype. In contrast, the D antigen is complex and may require an algorithm such as phenotype first then genotype only if weak D is identified in the transfusion service or genotype all D-negative individuals at a donor center. For the S antigen, the appropriate antisera may at times not be available; thus, genotyping could be used.

A second aspect of cost is determined by the need for repeat testing. Ideally, genotyping only needs to be performed once. Reasons to repeat genotyping are to ensure the results are correct and were entered correctly, but other quality assurance systems should be in place to prevent these errors. Another important consideration is that the genotyping information be transferable from institution to institution nationally and internationally, which would eliminate the need for repeat

testing of patients and blood donors. Genotyping likely could be provided in a cost-effective manner if appropriate algorithms are followed and repeat testing is minimized.

Gold Standard

The blood bank community relies on FDA approval for RBC typing reagents, which are currently used as the gold standard to identify the presence of an antigen. As polymorphisms are discovered through molecular technologies, there must be a continual connection back to the presence of an immunologic antigen; the RBC genotype must be connected to a RBC phenotype.

The gold standard for antibody screening is less clear, although all methods are based on hemagglutination. Throughout the years, multiple platforms have been developed to enhance and ease antibody detection, yet minimize false-positive reactions, defined as reactions seen but no alloantibody identified. The use of genotyped RBC reagents will aid in choosing RBCs from people who are homozygous for the gene encoding the required antigen and also to an extended phenotype to minimize false-positive reactions, which could be secondary to the presence of low-prevalence antigens. It has been proposed to use recombinant cell lines expressing a single protein to enhance and simplify antibody detection and identification.³ The use of improved reagents in conjunction with improved antibody screening platforms could improve the sensitivity and specificity of the antibody identification testing.

Who to Genotype

Secondary to cost and resource limitations, genotyping of donors and patients could be rolled out to a selected group first. For example, only donors who have demonstrated a commitment to become a long-term blood donor are genotyped so cost is not wasted on those who do not return and patients who demonstrate repeated need for transfusion or form multiple alloantibodies. However, the goal should be once genotyping is established in the reference laboratories of donor and transfusion centers, widespread genotyping should be conducted and applied universally.

Genotype for All Blood Groups

The cost of genotyping increases as the number of SNPs needed to be tested for increases, but the

incremental cost is low. Of the genotyping platforms available, they range in the number of SNPs and blood group antigens tested, but all test for the clinically significant RBC antigens. Outside the major clinically significant RBC antigens, recipients who lack a high-prevalence antigen are at high risk of antibody formation, whereas donors who have the presence of low-prevalence antigens may induce an immune response in the recipient. Therefore, genotyping panels should predict the presence of a low-prevalence antigen or the absence of a high-prevalence antigen, which can be performed by testing for a single SNP.

Food and Drug Administration Approval

In the United States, the FDA regulates medical devices and biologics under the Federal Food, Drug, and Cosmetic Act and Public Health Service Act, respectively. Currently, reagents and medical devices used in the immunohematology laboratory are regulated by the Federal Food, Drug, and Cosmetic Act and are considered class I and II devices depending on control requirements, performance standards, and additional patient tracking and postmarket information. Additional regulations will likely be imposed on new genotyping technologies, and the application process is extensive and includes clinical trials.

On September 25-26, 2006, in Bethesda, MD, a workshop cosponsored by the FDA/Center for Biologics Evaluation and Research, the Department of Health and Human Services/Office of Public Health and Science, and the National Institutes of Health/National Heart, Lung and Blood Institute discussed the current status of molecular methods in the field of immunohematology and addressed potential questions and concerns held by the FDA, which is highlighted in the July 2007 supplement of *Transfusion*. Specific questions included the amount of pre-market clinical trials to evaluate these molecular technologies, voluntary or mandatory usage of genotyping in only reference laboratories, transfusion services or both, and if this technology will become the universal standard. The workshop concluded that genotyping presents a promising future for the transfusion community; however, many questions remain unanswered and the development of standardization, clinical guidelines, controls, and proficiency testing needs to be

established before widespread use can be advocated.⁴⁵

Quality Assurance

For the purposes of quality assurance, proficiency testing should be performed biannually as mandated by the Clinical Laboratory Improvement Act. Multiple external proficiency testing programs are available for RBC antigen genotyping including *RHD* genotyping from the College of American Pathologists Molecular Genetics Laboratory survey, annual ISBT workshops including various patient situations, and an exchange program provided by the Consortium for Blood Group Genes. In addition, in-house comparison of serology to the molecular result can be performed. Recently, the results from the 2006 ISBT workshop were published, in which 6 samples that included transfusion-dependent patients; D, c, and K testing of fetal DNA from amniotic fluid; and prenatal *RHD* testing from pregnant D-negative woman plasma for fetal DNA were distributed to 41 laboratories throughout the world.⁴⁶ A high level of accuracy was obtained; however, suggestions for improvement included formation of defined terminology for reporting blood group alleles and genotypes and a need for more standards and standardization including controls, which are currently unavailable. The next ISBT workshop will be held in 2008 with the intent of distributing both relatively simple and more complex genotypes.

Personal Identification Issues

With the introduction of the Health Insurance Portability and Accountability Act Privacy Rule to the medical community in the United States in 2003, regulations regarding Protected Health Information have been implemented with the intent of protecting an individual's personal and medical information. The use of unique identifiers not linked to a particular individual is mandatory. The creation of a donor or patient database, which can be accessed by multiple institutions and individuals, leads to questions of patient confidentiality and identification. However, with the use of unique identifiers similar to those in use by the ARDP, anonymity could be maintained. In addition, there are also security issues and informed consent issues for the storage of DNA, especially if it remains linked to an individual.

Repeat Testing, New Mutations, and Rare Genotypes

With the introduction of molecular methods to the immunohematology laboratory, much has been discovered regarding RBC antigens especially of the identification of additional RBC antigen polymorphisms. As more individuals are genotyped, new mutations and rare genotypes will be uncovered. New alleles identified should be added to the blood group antigen gene mutation database maintained by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/projects/mhc/xslcgi.fcgi?cmd=bgmut/home>).³ The ISBT Working Party on Terminology for Red Cell Surface Antigens is addressing the need for a system for naming blood group alleles.

Need for Irradiation

Currently, phenotype-matched RBC products are used in the treatment of a selected group of patients, such as patients with SCD, without known occurrence of transfusion associated-graft versus host disease (TA-GVHD). Because there is a move to genotype-match more antigens between the donor and recipient, it is possible that HLA matching may parallel RBC matching, and the genetic differences between the donor and recipient will become less. This would theoretically lead to an increase risk of TA-GVHD, and irradiation may be necessary. However, there have been no published studies investigating the probability of HLA matching in parallel to RBC matching.

PROPOSED CLINICAL TRIALS

Phenotype-Matched RBCs Versus Genotype-Matched RBCs

A randomized control trial (RCT) should be done in patients with SCD to receive either phenotype-matched or genotype-matched RBCs. Such an RCT would demonstrate the feasibility of performing this testing and finding these products as well as demonstrate the clinical benefit (frequency of transfusion, alloimmunization rate, transfusion reaction rate, and product delay time).

Donor Genotyping Versus Phenotyping

A clinical trial should be done to investigate the concordance between genotyping and phenotyping to determine the future need of phenotyping. The goal of this trial would be to create the stepwise

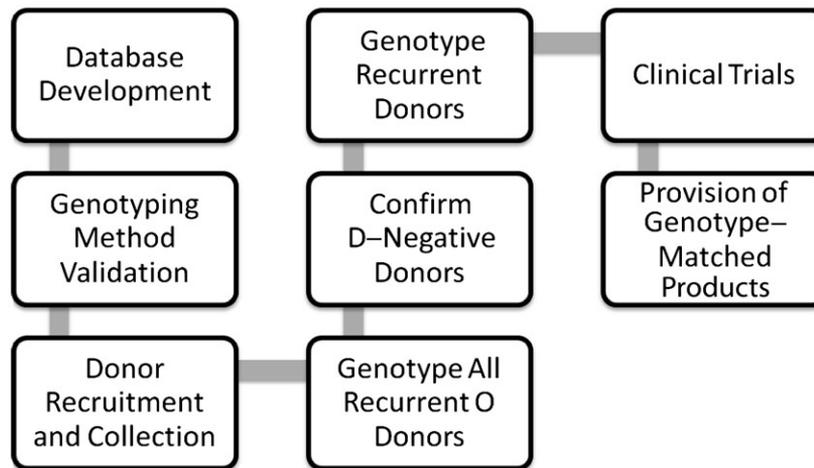


Fig 1. Stepwise process for the implementation of molecular testing in the donor center.

algorithm to ensure that antigen-negative products are identified correctly. A second goal would be to determine the need for the repeat genotyping of donors. To minimize costs, one time genotyping would be preferred if methods were in place to ensure the donor's identity.

Correlation Between HLA and RBC Antigens

To ensure that there is no increase risk of TA-GVHD, a clinical trial should be done to investigate pairs of unrelated RBC antigen-matched individuals to see if there is a HLA similarity and therefore a risk of TA-GVHD. This is important to

perform before implementing genotype-matching without the need for irradiated RBCs.

Genotyping to Eliminate the Need For Pretransfusion Testing

Small clinical trials have demonstrated that phenotype matching can prevent alloimmunization and therefore the need for antibody screening. A larger-scale trial is needed to evaluate the risks and benefits of eliminating or minimizing pretransfusion testing. The trial should follow alloimmunization rates after genotype-matched transfusions, product delays for locating matched

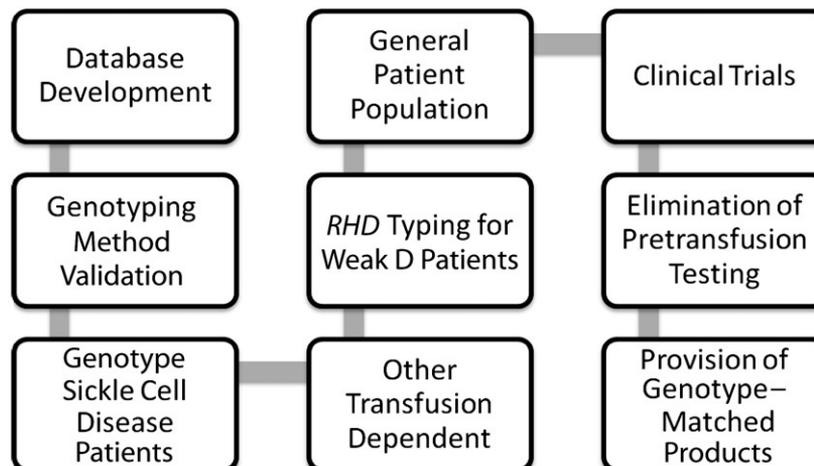


Fig 2. Stepwise process for the implementation of molecular testing in the transfusion service.

RBCs, transfusion reaction rates in comparison to decrease patient blood draws, product delays for testing, and cost.

RhIg and D-negative RBCs Administration

A clinical trial should be done with one group of patients following an algorithm for molecular *RHD* testing and appropriate transfusion practices based upon such results versus current standard of care. The outcome would be to look at anti-D formation rates in both groups.

IMPLEMENTATION OF MOLECULAR TESTING

In the Donor Center

Mass-scale genotyped blood donors could be entered in a shared database, a larger- and wider-scale version of the database currently in use by the ARDP. This would help the donor center find a particular product to distribute. (I) Repeat group O donors would be genotyped, (II) D-negative donors to ensure they are indeed D-negative, (III) all repeat donors, and (IV) eventually all donors (Fig 1). The database would allow recruitment of individuals whose blood type is needed as compared with general recruitment methods mostly used currently.

In the Transfusion Service

Molecular technologies have already been implemented in many hospital laboratories, for example, for the identification of human immunodeficiency virus, cytomegalovirus, hepatitis C, and chlamydia, and for HLA testing, and now should be introduced in a stepwise fashion in the transfusion service (Fig 2). The first phase of molecular testing in a transfusion service would be to create a detailed patient database with antibody and molecular antigen typing information. The initial population to test is patients with SCD who are already phenotyped and are receiving phenotype-matched RBCs. This would bring this process to a molecular level to produce improved RBC matching. The second population for whom to add molecular typing is other chronically transfused individuals and patients with warm autoantibodies. The third phase would be genotyping of *RHD* in patients with weak D phenotyping to aid in the determination of RhIg use and D-negative RBCs for transfusion. The fourth phase would be the genotyping of all patients. Ideally, these databases could be shared between institutions, or patients would receive this informa-

tion as a part of their Health Information System, which could be easily accessed when visiting another institution.

Database With Patient and Donor Information

Patients or donors move from one hospital or center to the next; this occurs particularly in many urban areas in the United States. This leads to repeat testing, and patient care can be delayed, or worse, incorrect products issued. An ideal database would have individual data present such that it could be accessed by both donor centers and hospitals (Fig 3). There would be a continuous cycle between donors and patients as donors become patients and patients become donors. On the transfusion service side, knowing the patient's blood type and previously identified antibodies streamlines antibody identification workup. The combined database would aid in matching/pairing donors to patients and limit donor exposure. It may also increase donor recruitment because one reason that potential donors do not donate is that the process is not seen as volunteering and is too impersonal.⁴⁷ This process of pairing a donor with a patient allows

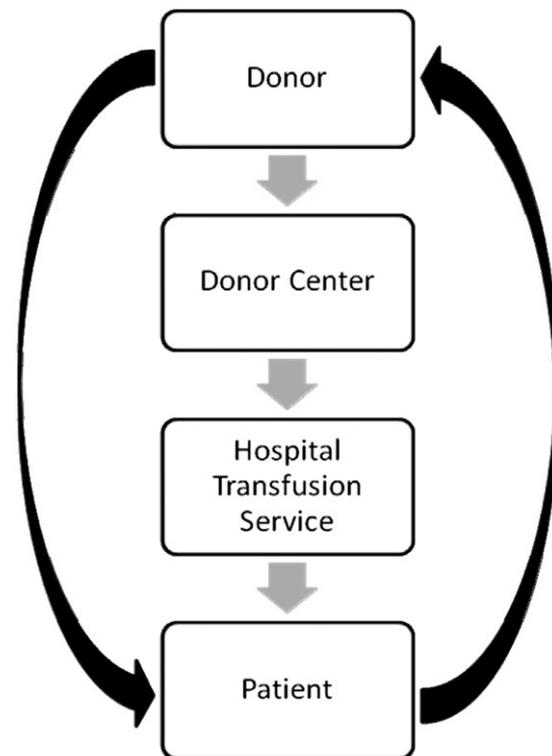


Fig 3. Donor and patient database development.

for personalization of the donation process. The merging of donor and patient databases would improve the care of patients plus give an additional inspiration for individuals to donate.

If extended genotype-matched products can be ensured for every transfusion, then the use of antibody screening would be minimal. It is unlikely that all antigens can be matched, but it is possible that the common clinically significant antigens (D, C, c, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, and s) could be matched for patients needing RBC transfusions, leaving only the low-prevalence antigens at risk to cause alloimmunization. In addition, patients should be identified for the lack of a high-prevalence antigen and, therefore, at high risk of forming the corresponding alloantibody; the logistics and likelihood of finding an appropriate antigen-matched product may make this approach difficult.

CONCLUSIONS

The transfusion community has moved to an exciting time where new molecular technologies

are emerging and being implemented. With these tools, patients can receive RBC antigen-matched products, which will decrease the number of DHTRs and decrease the time needed for antibody identification and finding the appropriate product. The application of molecular technologies will impact all aspects of transfusion medicine. Donor centers daily face problems due to the inability to find the proper antigen-negative products or to appropriately label a D-negative product, and the transfusion services struggle to identify alloantibodies, interpret ABO types in the face of an ABO typing discrepancy, correctly identify patients at risk for anti-D formation, and provide antigen-matched products to chronically transfused patients. There is a step-by-step process that donor centers and transfusion services must take to reach the goal of providing genotype-matched products to all and minimizing pretransfusion testing. Molecular testing has entered this field, and the transfusion medicine specialist must find the ideal and cost-effective way to use this powerful tool.

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