blood® Review Series

TRANSFUSION MEDICINE

Blood group genotyping

Connie M. Westhoff

Laboratory of Immunohematology and Genomics, New York Blood Center, New York, NY

Genomics is affecting all areas of medicine. In transfusion medicine, DNA-based genotyping is being used as an alternative to serological antibody-based methods to determine blood groups for matching donor to recipient. Most antigenic polymorphisms are due to single nucleotide polymorphism changes in the respective genes, and DNA arrays that target these changes have been validated by comparison with antibody-based typing. Importantly, the ability to test for antigens for which there are no serologic reagents is a major medical advance to identify antibodies and find compatible donor units, and can be life-saving. This review summarizes the evolving use and applications of genotyping for red cell and platelet blood group antigens affecting several areas of medicine. These include prenatal medicine for evaluating risk of fetal or neonatal disease and candidates for Rh-immune globulin; transplantation for bone marrow donor selection and transfusion support for highly alloimmunized patients and for confirmation of A2 status of kidney donors; hematology for comprehensive typing for patients with anemia requiring chronic transfusion; and oncology for patients receiving monoclonal antibody therapies that interfere with pretransfusion testing. A genomics approach allows, for the first time, the ability to routinely select donor units antigen matched to recipients for more than ABO/RhD to reduce complications. Of relevance, the growth of whole-genome sequencing in chronic disease and for general health will provide patients' comprehensive extended blood group profile as part of their medical record to be used to inform selection of the optimal transfusion therapy. (*Blood*. 2019;133(17):1814-1820)

Introduction

Blood typing by antibody-based methods has been the standard for determining ABO, Rh, and "extended" blood group antigens present on red blood cells (RBCs), now >300,[1] since the discovery of the ABO system. A blood group antigen is defined as polymorphism on RBCs (platelets and neutrophils) that differ between individuals and stimulates production of an immune antibody following exposure via pregnancy or blood transfusion. (A and B antigens, as carbohydrate epitopes on the RBCs, are an exception because the antibodies result from environmental microbe exposure.) The association of a specific polymorphism with antibody production is central to defining an antigen, as increased genetic variability in RBC membrane proteins will be revealed through genomic sequencing but these may not be immunogenic.

Commercial antibody reagents for ABO and Rh (D, C, c, E, e) and ~18 other common blood group antigen specificities are available; however, there are no typing reagents for many clinically significant antigens. The sensitivity and specificity of antibody reagents can vary and typing for "extended or minor" antigens has been challenging to automate because the carrier protein and/or carbohydrate are often part of transmembrane complexes, and recognition of the antigenic epitope often requires the native conformation be maintained. Antibody-based typing reagents have served the profession well for >50 years but the lack of automation for more than ABO/RhD, high cost of reagents and labor, and lack of specificities mean routine typing for transfusion, at least in the United States, has been limited to ABO and RhD as the most important blood groups. Table 1 compares antibody-based and DNA-based typing.

RBC genotyping approach and applications

Most blood group antigens, other than ABO and RhD, result from single nucleotide polymorphisms (SNPs), making genotype assay design and interpretation straightforward. This has resulted in the commercialization of DNA arrays that target 35 to 37 antigens in 12 blood group systems (not ABO or RhD) and human platelet antigen (HPA) panels targeting HPA 1 through 9, 11, and 15.[2-6] Implementation and licensing involved validating SNP-antigen associations with large numbers of samples tested in parallel and compared with antibody-based typing and gene sequencing. The field now has more than a decade of experience with the "prediction" of the RBC antigen phenotypes by DNA assays, and results are highly correlated with testing of the RBCs with a specific antibody. DNA arrays are primarily available in blood centers, or in large hospitals that also collect donor units, and used for high-throughput extended typing of donor samples. Most hospital transfusion service laboratories do not have the equipment and the dedicated environment required for

Table 1. Comparison of methods for blood group antigen typing

Antibody-based typing	DNA-based typing
≤1-h turnaround	24-h turnaround
Manual	Automated
Fresh RBCs required	Any cell source*
Existing equipment	Specialized equipment and environment
Direct detection of antigen expression	Indirect "predicted" antigen expression
Interference from transfused RBCs or bound IgG	No interference from transfused RBCs or bound IgG
No reagents for some clinically significant antigens	Type for any antigen whose genetic basis is known
Weak/variable antigen expression may be missed	Detection of weak antigen expression
Low resolution	High resolution possible

IgG, immunoglobulin G.

*Including fetal typing from cell-free DNA in maternal plasma.

DNA methods and patient samples are referred to blood centers for testing.

ABO and RhD

DNA arrays for extended RBC typing do not routinely include ABO and RhD, although for the majority of individuals these can be accurately determined by interrogation of multiple SNPs.[7,8] The problem is that a single mutation anywhere in an A or B allele or in an RHD allele can result in an inactive transferase (ie, a group O phenotype)[9] or absence of RhD (ie, an RhD^- phenotype). Because DNA arrays cannot target every nucleotide in the gene, a novel or uncommon mutation would be undetected, and the possibility of not getting the ABO or RhD correct is unacceptable. Because serologic ABO/RhD typing is fast, accurate, and relatively inexpensive, it is anticipated that genotyping will not be relied on as the sole means for determining ABO/RhD, in contrast to other blood group antigens. Nevertheless, ABO genotyping is increasingly being used for transplant registries, which routinely collect buccal swabs but not red cell samples, to aid in donor selection. ABO genotyping is also useful to resolve patient and blood donor typing discrepancies,[10,11] determine the original blood type of patients massively transfused to conserve group O donor inventories, or to determine the original blood type of transplant recipients by testing a buccal sample. ABO genotyping is also used to confirm A_2 subgroup in kidney donors who may have been transfused or whose RBCs give discordant reactivity in serologic testing with anti-A_1 reagents.

Pretransfusion compatibility testing

Current pretransfusion testing of the patient sample includes ABO and RhD typing and antibody screening to detect antibodies in the plasma to blood group antigens. If positive, the antibody target must be identified and further confirmed by typing patient RBCs to show they lack that antigen. Donor RBCs for transfusion must also be typed and shown to lack the antigen, and a compatibility test with donor RBCs and patient plasma is required.

Because patients who become immunized to RBC antigens are "responders" and have increased risk to make additional antibodies,[12] some proactive transfusion services perform an extended antigen type, especially if the patient is facing further transfusion. The goal is to determine common antigens the patient lacks and is at risk of producing immune antibody if exposed. Some may then choose to match patients with donors for additional antigens to avoid further antibody complications, depending on the situation. Even if not acted on initially, extended antigen typing expedites future pretransfusion testing by narrowing the number of different antibody specificities that must be ruled out to include only those antigens that the patient lacks. Extended antigen profiling is best done by genotyping because testing involves a single automated assay that has been shown to be more accurate and provide more information.[13] Testing only needs to be performed once when made part of the patient transfusion record. Table 2 summarizes situations in which DNA-based genotyping is advantageous compared with antibody-based methods.

Extended antigen matching

Antigen matching for ABO/RhD and K (a Kell system antigen) for female patients needing transfusion is common in several European countries and Australia to avoid hemolytic disease of the newborn because of sensitization to K, which is associated with neonatal morbidity and mortality. Avoiding alloimmunization for other Rh antigens (C, c, E, or e) is also routinely done in some European systems for women and patients receiving chronic transfusion. In the United States, prophylactic antigen matching for C, E, and K is becoming common for patients with sickle cell disease (SCD). Antigen matching for C, c, E, e, and K as well as Fya/b, Jka/b, and Ss is also increasingly being used for patients when compatibility cannot be demonstrated by routine testing because of the presence of warm autoantibodies or drug interference. This approach replaces the use of "least incompatible" blood for transfusion with donor units "antigen matched for clinically significant blood group antigens." This reduces the risk of delayed transfusion reactions and can circumvent additional alloimmunization, but also improves patient care and test turnaround time by eliminating the need for or reducing the frequency of repeat labor-intensive adsorptions to remove autoantibody (or drug antibody) to detect underlying specific RBC alloantibodies.

Monoclonal antibody interference in pretransfusion testing

Some monoclonal antibody drug therapies cause interference in pretransfusion testing. The most often encountered is anti-CD38 (daratumumab),[14,15] approved in 2015 for treatment of multiple myeloma. Additional anti-CD38 drug therapies (MOR202 and isatuximab) are now in clinical use, and clinical trials testing the efficacy of anti-CD38 in other conditions is an active area of research.[16] A monoclonal antibody targeting CD47 is also in phase 1 clinical trials.[17] In contrast to anti-CD38, which interferes in the indirect antiglobulin testing, anti-CD47 interferes with all phases of pretransfusion testing, including ABO typing.[18] Patients receiving anti-CD47 also have some degree of anemia and thrombocytopenia, which increases the possibility that they may require transfusions. Obtaining the patient's pretherapy

Table 2. Uses of DNA-based genotyping for transfusion medicine

Type patients for multiple antigens in a single assay
Type patients who have been recently transfused or RBCs coated with immunoglobulin
Type patients with autoimmune hemolytic anemia (to select antigen-negative RBCs for transfusion and adsorption of autoantibodies when searching for underlying alloantibodies)
Type patients receiving monoclonal antibody therapies that interfere with pretransfusion testing
Type RBCs when commercial antisera are not available
Type obstetric patients to identify weak D and partial D phenotypes to determine candidates for RhIg and to avoid use of limited RhD^- blood)
Resolve blood group typing discrepancies
Determine paternal zygosity for RHD and HPA
Type fetus to determine risk for HDFN or FNAIT
Accurate Rh antigen matching in SCD

RhIg, Rh immune globulin.

sample for baseline ABO/RhD and antibody screening and performing extended RBC phenotype or genotype before the start of monoclonal antibody therapies is recommended by AABB.[19] As discussed previously, the use of extended antigen-matched donor units for transfusion can reduce the number and complexity of repeat pretransfusion workups.

RBC genotyping in SCD

Alloimmunization is a serious complication of chronic transfusion, particularly for patients with SCD and β-thalassemia. Many programs attempt to prevent or reduce the risk of alloantibodies by transfusing donor units that are antigen matched for D, C, E, and K,[20-23,24] and some include $Fy^{a/b}$, $Jk^{a/b}$, and S.[25] Performing an extended antigen profile on patients with SCD before the first transfusion is recommended (American Society of Hematology committee on the development of guidelines for management of SCD, manuscript in preparation). This can be performed by serologic typing or by DNA methods, although genotyping improves accuracy and provides more information.[13] For example, RBC genotyping identifies the ~20% of patients with SCD whose RBCs type C^+, but who have altered C antigen. They should receive C^- donor units because it has been shown in several cohorts that one-third of these patients make anti-C if transfused with C^+ donor units.[23,26] Genotyping also identifies patients who lack high-prevalence antigens, and although prophylactic prevention is often not possible, having this information avoids misdiagnosis because the antibody in laboratory testing will appear to be a warm autoantibody. Genotyping also alerts the transfusion service to incompatibilities in the Dombrock (Doa/b, Joa,Hy), other Kell (Kpa/b, Jsa/b), other Rh (V/VS), and Colton (Coa/b), Yt (a/b), Lutheran (Lua/b), Diego (Dia/b), and Scianna (Sc1/2) systems. These antibodies can be clinically significant and life-threatening, but difficult to identify; no methods were previously available to type donors to find compatible units. Incompatibility in these systems should be considered when hemolysis of transfused RBCs is observed in the absence of detectable new antibodies.

Patients with SCD sometimes make Rh antibodies despite D, C, and E antigen matching by serology. These antibodies are found in multiply transfused patients whose RBCs are positive for the antigen by serologic typing or are negative for the antigen but have not been exposed to the antigen on donor RBCs as determined by serologic typing.[23,27] This occurs because Rh epitopes are complex and RH genes in individuals of African black background are diverse. Consequently, both the patients and the African American donors often used to transfuse them have RHD and RHCE alleles encoding Rh epitopes whose serologic reactivity is not straightforward.[27-29] Overall, ~6% of African American RhD^+ patients with SCD are at risk for clinically significant anti-D, ~21% of C^+ patients are at risk for anti-C, and 21% are at risk for anti-c or anti-e when prophylactic matching is based on serology.[27] Similar to HLA, some patients with SCD are not Rh antigen matched sufficiently by serologic typing to avoid alloimmunization; high-resolution typing is then required. Importantly, RH allele frequencies are similar between patients with SCD and African American blood donors, suggesting higher resolution Rh matching by RH genotyping would be potentially feasible in the future and enable better use of African American blood donor inventories.[27]

RBC genotyping in bone marrow transplantation

Although most antibodies to RBCs including ABO are not a barrier to engraftment, alloantibodies can cause complications. The increasing use of reduced myeloablative conditioning results in mixed chimerism, with persistence of both recipient and donor-derived lymphocytes and recipient plasma cells. Existing antibodies can cause delayed erythropoiesis or hemolysis of engrafting donor RBCs, and new antibodies (whether donor- or patient-derived) can complicate transfusion support and recovery. For transplant patients who have existing RBC alloantibodies, genotyping both the donor and recipient for extended blood group antigens and evaluating donor/recipient compatibility before transplantation proactively informs donor selection and transfusion support. If several HLA-identical donors are eligible (ie, siblings in a related transplant), RBC genotyping can guide donor selection.[30] Pretransplant donor/recipient evaluation is particularly relevant for patients with SCD undergoing transplantation who are highly RBC alloimmunized, which is an increasingly common situation.[31] Posttransplantation, genotyping of the recipient peripheral blood sample and buccal cells can be done to determine the origin of new antibodies (ie, donor- or recipient-derived) to inform selection of units for transfusion to provide the best transfusion support.

Prenatal testing (FNAIT, HDFN, and RHD typing)

The ability to determine red cell or platelet antigens by DNA-based genotyping advances diagnosis and evaluation of hemolytic disease of the fetus and newborn (HDFN) and fetal and neonatal alloimmune thrombocytopenia (FNAIT) when the mother has antibodies to either RBC or platelet antigens. The

most common cause of HDFN is immunization to RhD or to K antigen. Twenty-eight HPAs have been characterized, but incompatibility in HPA-1 accounts for ~80% of all FNAIT cases.[32,33]

Paternal testing

Determining the paternal genotype is key to assessing the risk for complications. If the father tests antigen positive, the paternal gene copy number (zygosity) is determined. If the father is homozygous for the gene, all of the children will be antigen positive and monitoring of the pregnancy is required. If the father is heterozygous, the fetus has a 50% chance of being unaffected.

Fetal testing

If the father has a single copy of the gene encoding the antigen (or paternity is uncertain or unknown), fetal DNA from cells obtained by amniocentesis can be tested to determine the antigen status of the fetus. If the fetus is antigen negative, the mother need not undergo invasive and costly monitoring or receive immune-modulating agents. If the fetus is antigen positive, pregnancy management includes monitoring and treatment, if needed, with intrauterine blood transfusion. More recently, noninvasive testing of cell-free fetal-derived DNA present in maternal plasma is being used to determine the antigen status of the fetus to minimize adverse events associated with amniocentesis.[34] Cell-free fetal DNA derived from placental trophoblasts is present by 5 weeks of gestation and increases throughout pregnancy. Noninvasive testing is performed in Europe to type the fetus for most clinical blood group antigens (D, c, C, E, e, K, HPA-1a), and invasive procedures for fetal blood group typing have become obsolete in some countries.[35,36] In contrast, in the United States, questions remain about the clinical utility of noninvasive testing to avoid adverse events associated with amniocentesis, and test availability is limited.[37,38]

RHD genotyping and RhIg

For RhD$^-$ mothers, prophylaxis with RhIg effectively mitigates the risk of immunization and production of anti-D. Postnatal immunoprophylaxis is given based on the RhD blood type of the newborn, whereas the antenatal dose is routinely administered to all RhD$^-$ women as the fetal blood type is not known. However, it is now routine practice in several European countries to use noninvasive testing of cell-free fetal DNA in maternal plasma to determine fetal RhD type and avoid unnecessary administration of antenatal RhIG to the ~38% to 40% of women who carry an RhD$^-$ fetus.[38] The results are also used to guide postnatal prophylaxis, and no fetal cord blood serology is performed.[39-42] This approach has been implemented in The Netherlands,[39] Denmark,[43,44] Sweden,[45] United Kingdom,[40] and France[42] to avoid unnecessary administration of RhIg, a human blood product with limited supply.

One of the challenges in identifying women who are candidates for RhIg concerns RhD typing of the maternal RBCs. Approximately 1% to 6% of individuals, depending on their ethnic background, have inherited alleles encoding altered RhD, and the RBCs type by antibody-based methods as RhD$^+$ or RhD$^-$, or much weaker than expected, depending on the method and/or reagent used for typing. Some of these, but not all, lack major epitopes of RhD (called partial D phenotypes) and hence are at risk for anti-D directed to RhD epitopes missing on their RBCs. It is generally accepted that women with partial D phenotypes benefit from receiving RhIg prophylaxis if they deliver an RhD$^+$ fetus, but which women are at risk of clinically significant anti-D cannot be identified by antibody-based typing. Transfusion services often err on the side of caution and treat women with RBCs that type weaker than expected (<2+ reactivity) or RBCs that type positive with some reagents and negative with others, as RhD$^-$. This results in the unnecessary use of RhIg and of RhD$^-$ blood. *RHD* genotyping can discriminate partial D phenotypes at risk for clinically significant anti-D; in 2015, a workgroup representing a number of clinical standard-setting organizations recommended that *RHD* genotyping be incorporated in the management of pregnant women.[46,47] Experience with *RHD* genotyping is illustrated in Figure 1. Approximately 45% to 50% of women of mixed ethnic groups with weaker than expected RhD typing were not at risk for anti-D and not candidates for RhIg. Limitations remain in that some women who would benefit from RhIg are missed (RBC type strongly RhD$^+$), and some women in the absence of an indirect antiglobulin test method appear to be RhD$^-$ and still receive unnecessary RhIg. It is anticipated that in the future it will be routine to determine the RhD type of all pregnant women by *RHD* genotyping to accurately guide transfusion and RhIg prophylaxis.

Genotyping for blood donors

Confirming RhD type donors

Donor centers must perform a test for weak expression of RhD to avoid labeling a blood product as RhD$^-$ that might result in anti-D in response to transfused RBCs. Approximately 0.1% of donor RBCs have very weak RhD$^+$ expression not detected with antibody reagents and are labeled as RhD$^-$ for transfusion. Although the clinical significance has not been well documented, donor RBCs with very weak RhD expression have been associated with alloimmunization.[48] *RHD* genotyping would improve donor testing by confirming RhD$^-$ typing.

Resolving donor ABO to retain donors

Healthy donors sometime have depressed ABO antigens (subgroups) or low titers of natural ABO antibodies. The products cannot be labeled for transfusion because the RBC and plasma ABO typing appears to be discordant and products are often discarded. *ABO* genotyping can determine the donor's inherited ABO type, and although testing is not approved for labeling at the present time, DNA methods offer the potential to be a "confirmatory test." This would avoid discarding safe and effective donor products and retain donors with depressed antibody titers whose products are actually superior when out-of-ABO group plasma and platelet transfusions must be used.[11]

NGS

DNA arrays interrogate only a limited number of SNPs. Comprehensive analysis of all blood groups of interest in a single assay is not currently possible (ie, there are 360 defined RBC antigens and 33 platelet antigens encoded by genetic changes in 45 RBC and 6 platelet genes). More than 2000 alleles have been identified associated with differences in RBC phenotype or with antibody production. It is possible to determine all RBC and platelet antigens of interest from next-generation sequencing (NGS) of whole genomes or exomes or by targeting the specific blood group loci. A number of publications over the past 6 years have shown that NGS provides adequate coverage of the genes

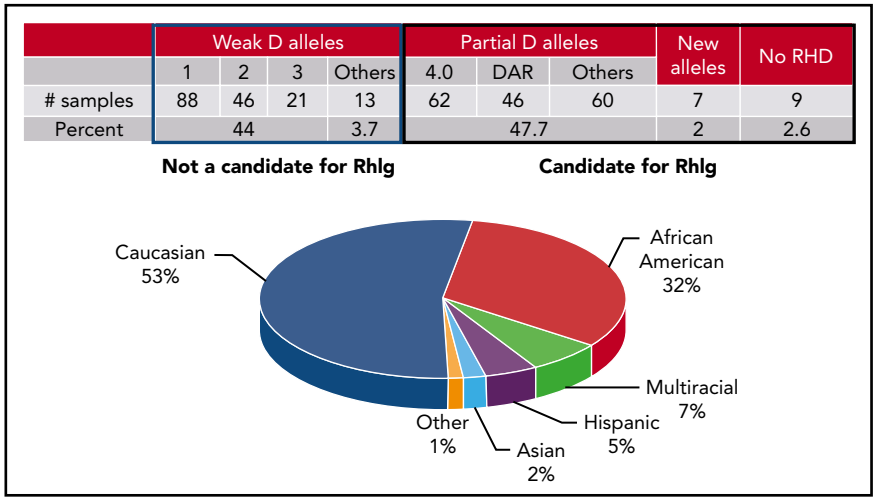

Figure 1. Results of *RHD* genotyping of 352 pregnant women of diverse ethnic backgrounds presenting with discrepant or weaker than expected RhD typing of their red cells. Approximately 48% are not at risk for clinically significant immunization and RhIg would be unnecessary.

for blood group extended antigen typing,[28,49-54] and for RH genotyping in SCD.[28] The strength of this approach lies in the ability to detect all polymorphisms including null alleles, novel mutations, and complex gene rearrangements, and to perform copy number analysis. This comprehensive approach has advantages to resolve complex cases with the capability of identifying rare and novel blood group variants that would otherwise be unrecognized,[55,56] particularly when the antibody specificity is uncertain or, importantly, when transfused RBC survival is compromised in the absence of detectable antibody. Efforts to create an algorithm to directly interpret data from NGS and to generate a report that includes the extended profile are being developed and validated.[54] As the cost of NGS continues to drop, patient genome sequencing as part of clinical care will become more commonplace. Secondary analysis of NGS data for patients needing transfusion therapy would represent a cost-effective and practical use of this information to select blood products and improve transfusion safety.

Changing practice, costs, and reimbursement

Alloimmunization has been an accepted risk of blood transfusion in the absence of mitigation strategies. It is generally accepted that some level of antigen matching of the patient and donor beyond ABO/RhD would improve outcomes and reduce alloimmunization as is practiced in a number of countries with government-sponsored health care systems. As discussed, the primary focus in those countries for many years has been on prevention of sensitization for females of childbearing potential and for patients needing chronic transfusion. In the United States, providing some level of antigen matching is increasing being applied for patients with SCD or thalassemia, but what is necessary to broaden the approach in the era of precision medicine using information available through genomics? Barriers include lack of reimbursement for prevention, the regulated environment of transfusion medicine practice, and the cost structure of antigen-typed donor RBCs provided by blood centers. Providing donor units typed for more than ABO/RhD has historically been performed by labor-intensive manual serologic methods, which is reflected in the current cost structure. With DNA-based genotyping, extended antigen typing of donors is becoming practical, more economical, and readily available.[6,57-60] A change in practice to prevention and precision transfusion will require products to be labeled at the donor center and be available at the hospital, and, in the absence of reimbursement, be shown to reduce hospital labor, costs, and improve turnaround time and patient care.[60] The cost of patient testing can be reduced by referring samples to the donor center using a centralized testing model, and testing need only be performed 1 time and made part of the patient transfusion record.

Cost-benefit analysis is often challenging and reimbursement impacts efforts to improve both transfusion medicine and prenatal practice. Specifically, the use of *RHD* genotyping for accurate RhD typing of pregnant women to guide selection of blood for transfusion and RhIg candidacy has been shown to be cost neutral or cost saving over treating pregnant women as RhD$^-$ and using RhIg when *RHD* genotyping costs are ~$258.[61] In kind, non-invasive cell-free fetal testing from the maternal plasma to restrict antenatal and postnatal RhIg to only those RhD$^-$ women who carry an RhD+ fetus has been shown to be cost-effective in the Netherlands.[39] In the United States, using this approach has been hampered by the high cost and limited test availability associated with patent and licensing.[62] However, this approach is now common in many national health care systems and will become more readily available and accepted in the future.[36]

Summary

Blood transfusion is one of the most commonly administered therapies in clinical medicine. Current pretransfusion testing includes matching the patient and donor for ABO and RhD using approaches that have served the profession well, but that have not materially changed for >60 years. It is an exciting time in transfusion medicine because the field is poised to benefit from a genomics approach not only for recipient and donor compatibility determination, but for donor recruitment, donor health, product storage characteristics, and characterization of the metabolomic components of transfusion. The development of a transfusion medicine array is under way for study of transfusion-relevant variation in both donor and recipient populations, including outcomes funded by National Heart, Lung, and Blood Institute Recipient Epidemiology Donor Evaluation Study III.[63] The array targets >800 000 markers

and was developed to allow the genome wide analysis of diverse populations to study transfusion medicine–specific variation, including RBC and platelet structure and function, HLA, HPA, iron metabolism, sickle cell trait, glucose-6-phosphate-dehydrogenase deficiency, and others. In conclusion, genomic approaches promise to fundamentally change the way donors are recruited, units are stored, and products are selected for transfusion.

Authorship

Contribution: C.M.W. researched the manuscript and wrote the review.

Conflict-of-interest disclosure: C.M.W. declares no competing financial interests.

Correspondence: Connie M. Westhoff, Laboratory of Immunohematology and Genomics, New York Blood Center, 310 East 67th St, New York, NY 10065; e-mail: cwesthoff@nybloodcenter.org.

Footnote

Submitted 20 November 2018; accepted 24 January 2019. Prepublished online as *Blood* First Edition paper, 26 February 2019; DOI 10.1182/blood-2018-11-833954.

REFERENCES

1. Storry JR, Clausen FB, Castilho L, et al. International Society of Blood Transfusion Working Party on Red Cell Immunogenetics and Blood Group Terminology: report of the Dubai, Copenhagen and Toronto meetings. *Vox Sang*. 2019;114(1):95-102.

2. Beiboer SH, Wieringa-Jelsma T, Maaskant-Van Wijk PA, et al. Rapid genotyping of blood group antigens by multiplex polymerase chain reaction and DNA microarray hybridization. *Transfusion*. 2005;45(5):667-679.

3. Denomme GA, Van Oene M. High-throughput multiplex single-nucleotide polymorphism analysis for red cell and platelet antigen genotypes. *Transfusion*. 2005;45(5):660-666.

4. Montpetit A, Phillips MS, Mongrain I, Lemieux R, St-Louis M. High-throughput molecular profiling of blood donors for minor red blood cell and platelet antigens. *Transfusion*. 2006;46(5):841-848.

5. Hashmi G, Shariff T, Zhang Y, et al. Determination of 24 minor red blood cell antigens for more than 2000 blood donors by high-throughput DNA analysis. *Transfusion*. 2007;47(4):736-747.

6. Shehata N, Denomme GA, Hannach B, Banning N, Freedman J. Mass-scale high-throughput multiplex polymerase chain reaction for human platelet antigen single-nucleotide polymorphisms screening of apheresis platelet donors. *Transfusion*. 2011;51(9):2028-2033.

7. Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O2 versus A/O2 discriminating nucleotide substitution at the ABO locus. *Vox Sang*. 1995;69(3):242-247.

8. Westhoff CM. Rh complexities: serology and DNA genotyping. *Transfusion*. 2007;47(1 Suppl):17S-22S.

9. Chester MA, Olsson ML. The ABO blood group gene: a locus of considerable genetic diversity. *Transfus Med Rev*. 2001;15(3):177-200.

10. Flegel WA. Molecular genetics and clinical applications for RH. *Transfus Apheresis Sci*. 2011;44(1):81-91.

11. Westhoff CM. The concept of "confirmatory testing" of donors for ABO and RhD. *Transfusion*. 2013;53(11 Suppl 2):2837-2839.

12. Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? *Transfusion*. 2006;46(4):630-635.

13. Casas J, Friedman DF, Jackson T, Vege S, Westhoff CM, Chou ST. Changing practice: red blood cell typing by molecular methods for patients with sickle cell disease. *Transfusion*. 2015;55(6 Pt 2):1388-1393.

14. Chapuy CI, Nicholson RT, Aguad MD, et al. Resolving the daratumumab interference with blood compatibility testing. *Transfusion*. 2015;55(6 Pt 2):1545-1554.

15. Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. *Transfusion*. 2015;55(6 Pt 2):1555-1562.

16. National Institutes of Health, US National Library of Medicine,Clinicaltrials.gov. Clinical trials on anti-CD38. Available at: https://clinicaltrials.gov/ct2/results?cond=&term=anti-CD38&cntcy=&state=&city:=&dist=. Accessed 1 January 2019.

17. National Institutes of Health, US National Library ofMedicineClinicaltrials.gov. Clinical trials on *CD47*. Available at: https://clinicaltrials.gov/ct2/results?cond=&term=CD47&cntly=&state=&city=& dist=. Accessed 1 January 2019.

18. Velliquette RW, Aeschlimann J, Kirkegaard J, et al. Monoclonal anti-CD47 interference in red cell and platelet testing transfusion. *Transfusion*. 2019;59(2):730-737.

19. AABB. Association bulletin #16-02. Available at: http://www.aabb.org/programs/publications/bulletins/Documents/ab16-02.pdf. Accessed 1 January 2019.

20. Tahhan HR, Holbrook CT, Braddy LR, Brewer LD, Christie JD. Antigen-matched donor blood in the transfusion management of patients with sickle cell disease. *Transfusion*. 1994;34(7):562-569.

21. Vichinsky EP, Luban NL, Wright E, et al; Stroke Prevention Trail in Sickle Cell Anemia. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. *Transfusion*. 2001;41(9):1086-1092.

22. Castro O, Sandler SG, Houston-Yu P, Rana S. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. *Transfusion*. 2002;42(6):684-690.

23. Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DF, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood*. 2013;122(6):1062-1071.

24. O'Suoji C, Liem RI, Mack AK, Kingsberry P, Ramsey G, Thompson AA. Alloimmunization in sickle cell anemia in the era of extended red cell typing. *Pediatr Blood Cancer*. 2013;60(9):1487-1491.

25. Lasalle-Williams M, Nuss R, Le T, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). *Transfusion*. 2011;51(8):1732-1739.

26. Tournamille C, Meunier-Costes N, Costes B, et al. Partial C antigen in sickle cell disease patients: clinical relevance and prevention of alloimmunization. *Transfusion*. 2010;50(1):13-19.

27. Chou ST, Evans P, Vege S, et al. *RH* genotype matching for transfusion support in sickle cell disease. *Blood*. 2018;132(11):1198-1207.

28. Chou ST, Flanagan JM, Vege S, et al. Whole-exome sequencing for *RH* genotyping and alloimmunization risk in children with sickle cell anemia. *Blood Adv*. 2017;1(18):1414-1422.

29. Reid ME, Halter Hipsky C, Hue-Roye K, Hoppe C. Genomic analyses of RH alleles to improve transfusion therapy in patients with sickle cell disease. *Blood Cells Mol Dis*. 2014;52(4):195-202.

30. Fasano RM, Monaco A, Meier ER, et al. RH genotyping in a sickle cell disease patient contributing to hematopoietic stem cell transplantation donor selection and management. *Blood*. 2010;116(15):2836-2838.

31. Allen ES, Nelson RC, Flegel WA. How we evaluate red blood cell compatibility and transfusion support for patients with sickle cell disease undergoing hematopoietic progenitor cell transplantation. *Transfusion*. 2018;58(11):2483-2489.

32. Turner ML, Bessos H, Fagge T, et al. Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. *Transfusion*. 2005;45(12):1945-1956.

33. Williamson LM, Hackett G, Rennie J, et al. The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PIA1, Zwa) as determined by antenatal screening. *Blood*. 1998;92(7):2280-2287.

34. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet*. 1997;350(9076):485-487.

35. Scheffer PG, van der Schoot CE, Page-Christiaens GC, de Haas M. Noninvasive fetal blood group genotyping of rhesus D, c, E and of K in alloimmunised pregnant women: evaluation of a 7-year clinical experience. *BJOG*. 2011;118(11):1340-1348.

36. Scheffer PG, Ait Soussan A, Verhagen OJ, et al. Noninvasive fetal genotyping of human platelet antigen-1a. *BJOG*. 2011;118(11): 1392-1395.

37. Daniels G, Finning K, Lozano M, et al. Vox Sanguinis International Forum on application of fetal blood grouping. *Vox Sang*. 2018; 113(2):e26-e35.

38. Daniels G, Finning K, Lozano M, et al. Vox Sanguinis International Forum on application of fetal blood grouping: summary. *Vox Sang*. 2018;113(2):198-201.

39. de Haas M, Thurik FF, van der Ploeg CP, et al. Sensitivity of fetal RHD screening for safe guidance of targeted anti-D immunoglobulin prophylaxis: prospective cohort study of a nationwide programme in the Netherlands. *BMJ*. 2016;355:i5789.

40. Soothill PW, Finning K, Latham T, Wreford-Bush T, Ford J, Daniels G. Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. *BJOG*. 2015; 122(12):1682-1686.

41. Clausen FB, Jakobsen TR, Rieneck K, et al. Pre-analytical conditions in non-invasive prenatal testing of cell-free fetal RHD. *PLoS One*. 2013; 8(10):e76990.

42. Benachi A, Delahaye S, Leticee N, Jouannic JM, Ville Y, Costa JM. Impact of non-invasive fetal RhD genotyping on management costs of rhesus-D negative patients: results of a French pilot study. *Eur J Obstet Gynecol Reprod Biol*. 2012;162(1):28-32.

43. Clausen FB, Rieneck K, Krog GR, Bundgaard BS, Dziegiel MH. Noninvasive antenatal screening for fetal RHD in RhD negative women to guide targeted anti-D prophylaxis. *Methods Mol Biol*. 2019;1885:347-359.

44. Clausen FB, Steffensen R, Christiansen M, et al. Routine noninvasive prenatal screening for fetal RHD in plasma of RhD-negative pregnant women-2 years of screening experience from Denmark. *Prenat Diagn*. 2014; 34(10):1000-1005.

45. Wikman AT, Tiblad E, Karlsson A, Olsson ML, Westgren M, Reilly M. Noninvasive single-exon fetal RHD determination in a routine screening program in early pregnancy. *Obstet Gynecol*. 2012;120(2 Pt 1):227-234.

46. Sandler SG, Flegel WA, Westhoff CM, et al; College of American Pathologists Transfusion Medicine Resource Committee Work Group. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. *Transfusion*. 2015;55(3):680-689.

47. Haspel RL, Westhoff CM. How do I manage Rh typing in obstetric patients? *Transfusion*. 2015;55(3):470-474.

48. Flegel WA, von Zabern I, Wagner FF. Six years' experience performing RHD genotyping to confirm D- red blood cell units in Germany for preventing anti-D immunizations. *Transfusion*. 2009;49(3):465-471.

49. Fichou Y, Mariez M, Le Maréchal C, Férec C. The experience of extended blood group genotyping by next-generation sequencing (NGS): investigation of patients with sickle-cell disease. *Vox Sang*. 2016;111(4): 418-424.

50. Lane WJ, Westhoff CM, Uy JM, et al; MedSeq Project. Comprehensive red blood cell and platelet antigen prediction from whole genome sequencing: proof of principle. *Transfusion*. 2016;56(3):743-754.

51. Möller M, Jöud M, Storry JR, Olsson ML. Erythrogene: a database for in-depth analysis of the extensive variation in 36 blood group systems in the 1000 Genomes Project. *Blood Adv*. 2016;1(3):240-249.

52. Fichou Y, Férec C. NGS and blood group systems: state of the art and perspectives. *Transfus Clin Biol*. 2017;24(3):240-244.

53. Schoeman EM, Lopez GH, McGowan EC, et al. Evaluation of targeted exome sequencing for 28 protein-based blood group systems, including the homologous gene systems, for blood group genotyping. *Transfusion*. 2017;57(4):1078-1088.

54. Lane WJ, Westhoff CM, Gleadall NS, et al; MedSeq Project. Automated typing of red blood cell and platelet antigens: a whole-genome sequencing study. *Lancet Haematol*. 2018;5(6):e241-e251.

55. Montemayor-Garcia C, Westhoff CM. The "next generation" reference laboratory? *Transfusion*. 2018;58(2):277-279.

56. Schoeman EM, Roulis EV, Liew YW, et al. Targeted exome sequencing defines novel and rare variants in complex blood group serology cases for a red blood cell reference laboratory setting. *Transfusion*. 2018;58(2):284-293.

57. Klein HG, Flegel WA, Natanson C. Red blood cell transfusion: precision vs imprecision medicine. *JAMA*. 2015;314(15):1557-1558.

58. Flegel WA, Gottschall JL, Denomme GA. Implementing mass-scale red cell genotyping at a blood center. *Transfusion*. 2015;55(11): 2610-2615, quiz 2609.

59. Shafi H, Abumuhor I, Klapper E. How we incorporate molecular typing of donors and patients into our hospital transfusion service. *Transfusion*. 2014;54(5):1212-1219.

60. Westhoff C, Ness PM. The promise of extended donor antigen typing. *Transfusion*. 2015;55(11):2541-2543.

61. Kacker S, Vassallo R, Keller MA, et al. Financial implications of RHD genotyping of pregnant women with a serologic weak D phenotype. *Transfusion*. 2015;55(9):2095-2103.

62. Hawk AF, Chang EY, Shields SM, Simpson KN. Costs and clinical outcomes of noninvasive fetal RhD typing for targeted prophylaxis. *Obstet Gynecol*. 2013;122(3):579-585.

63. Guo Y, Busch MP, Seielstad M, et al; National Heart, Lung, and Blood Institute Recipient Epidemiology Donor Evaluation Study (REDS)-III. Development and evaluation of a transfusion medicine genome wide genotyping array. *Transfusion*. 2019;59(1): 101-111.

2019 133: 1814-1820
doi:10.1182/blood-2018-11-833954 originally published online February 26, 2019

Blood group genotyping

Connie M. Westhoff

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.