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On Our Cover

Girl with a Red Hat (1666) by Johannes Vermeer is an example of a tronie, derived from Dutch meaning “face” and referring to a stylized work common to the Dutch Golden Age that, distinct from formal portrait painting, featured an anonymous model with an exaggerated expression or depicted as an iconic character. Girl with a Red Hat employs bold, rich colors, and the girl's eye-catching hat and robe and informal turn to face the viewer are striking and engaging. Vermeer created only two other panel works, and the painting's flamboyance and use of wood as a support medium have caused some to question its attribution, though dendrological analysis as well as technique considerations favor Vermeer. One of Vermeer's more famous works, at just 9 × 7⅞ inches, Girl with a Red Hat is also one of his smallest.

Small integral membrane protein 1 (SMIM1) is the subject of an article in this issue.

David Moolten, MD
Hematologic complications in a patient with *Glycine soja* polyagglutination following fresh frozen plasma transfusion


Polyagglutination is a rare and underdiagnosed condition, characterized by agglutination of red blood cells (RBCs) with almost all ABO-compatible adult sera. Polyagglutination can occur when a cryptantigen is exposed on RBCs via microbial enzyme activity. Because nearly all adults naturally produce antibodies against cryptantigens, transfusion of plasma can cause unexpected hemolysis and hematologic complications, such as thrombocytopenia and disseminated intravascular coagulation, in patients whose cryptantigens are exposed. We report a case of *Glycine soja* polyagglutination occurring in a 60-year-old African-American man with disseminated methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Prior to transfusion, the patient developed severe anemia of unknown etiology. Following transfusion of 3 units of fresh frozen plasma (FFP), his RBC count could not be determined for 24 days because of RBC agglutination in his blood sample. In addition, the FFP transfusion correlated with the rapid development of severe, transfusion-refractory thrombocytopenia and anemia. The perplexed clinical team consulted the blood bank. A direct antiglobulin test demonstrated 1+ mixed-field reactivity with both monoclonal anti-IgG and anti-C3d. Lectin panel testing showed reactivity with only *Glycine soja*, confirming the condition. Subsequently, plasma components were avoided, and RBC and platelet (PLT) components were washed prior to transfusion. After a 44-day hospitalization involving the transfusion of 22 units of RBCs and 13 units of PLTs, the patient was discharged to a long-term care facility. The patient’s confounding hematologic complications can best be explained by polyagglutination, which developed secondary to the severe MRSA infection. The FFP transfusion likely passively transferred antibodies that bound to the patient’s RBC cryptantigens, leading to RBC agglutination and anemia. The development of severe thrombocytopenia may be related to cryptantigen exposure on the patient’s PLTs. Although difficult to identify, polyagglutination needs to be recognized as a potential cause of hematologic complications following FFP transfusion in a patient with *Glycine soja* polyagglutination, a rarely described condition. Immunohematology 2017;33:51–55.

Key Words: unclassified polyagglutination, *Glycine soja*, *Staphylococcus aureus*, lectin, minor crossmatch, T activation

Polyagglutination describes the agglutination of red blood cells (RBCs) that occurs with nearly all compatible adult sera.\(^1\,^2\) It arises through an alteration of glycoprotein moieties on the RBC membrane. This condition is usually secondary to infection\(^1\,^2\) rather than to a congenital or somatic mutation. T activation is the most common form of microbial-induced polyagglutination and serves as a prototypical example (Fig. 1).\(^3\) Antibodies against cryptantigens are naturally occurring and are usually IgM.\(^4\) The patient’s own antibodies may mediate hemolysis.\(^4\) However, it is more common for hemolysis to occur after the transfusion of blood components that contain plasma.\(^5\,^6\) Thrombocytopenia may develop because of the presence of the cryptantigen on platelets (PLTs).\(^8\,\,^9\) In addition, coagulopathies, such as disseminated intravascular coagulation (DIC), often occur.\(^5\,\,^10\)

![Fig. 1](attachment:image.png) Microbial enzymes such as sialidases (circle sector shape) can enzymatically remove N-acetylneuraminic acid (triangles) from RBC antigens. This action exposes normally hidden cryptantigens, such as T (shown on the right), which can subsequently bind anti-T. This particular type of polyagglutination is known as T activation. This form is often described in pediatric patients with necrotizing enterocolitis or atypical hemolytic uremic syndrome caused by *Streptococcus pneumoniae*. T is also present on platelets and glomerular endothelial cells. The circle designates α-galactose. The square designates N-acetyl-β-galactosamine. R = Remainder of molecule; RBC = red blood cell.

In the past, polyagglutination was readily detected by blood centers when ABO typing resulted in ABO discrepancies.\(^8\,\,^11\) This finding occurred because human-source sera containing antibodies against cryptantigens were used for blood typing. The transition to monoclonal antibodies for blood typing
eliminated these ABO discrepancies. In addition, most blood centers do not routinely screen for polyagglutination. Therefore, polyagglutination is becoming under-recognized. To identify polyagglutination, the condition must be suspected by either clinicians or blood bank personnel, and additional testing must then be performed. Additional features seen in polyagglutination include a C3-positive direct antiglobulin test (DAT) and/or a reverse ABO typing discrepancy.

Lectin panel testing should be used to properly identify and classify polyagglutination. Lectins are proteins that bind to carbohydrate antigens. Lectin panel testing is not available in most blood banks, although this testing is performed by reference laboratories. Alternatively, polyagglutination can be more easily detected by demonstrating RBC agglutination with nearly all compatible adult sera. In rare cases, RBCs from patients with polyagglutination are not truly polyagglutinable, and polyagglutination can only be diagnosed using lectins.

Suspected polyagglutination may prompt the use of specialized transfusion protocols to minimize the passive transfer of antibodies targeting cryptantigens. Protocols involve avoiding plasma components and washing units of RBCs and PLTs. If plasma components must be given, the least incompatible units, as determined by minor crossmatch, should be selected. In addition, blood components should be transfused slowly with careful monitoring of the patient. For critically ill patients, plasma exchange using albumin as the replacement fluid and RBC exchange using washed RBCs have been reported as beneficial. Nevertheless, these transfusion practices are not universally accepted because of skepticism about a causal relationship between polyagglutination and hemolysis. In addition, some physicians note risks associated with avoiding therapeutic blood components and with the time delay caused by washing. In addition, washing can lead to bacterial contamination, the loss of 20 percent of RBCs, the loss of 25 percent of PLTs, and impaired hemostatic function of PLTs. Also, reports of uneventful transfusion of plasma components have led some physicians to discount the potential risks of transfusion.

Case Report

A 60-year-old African-American man with a past medical history of epileptic seizures developed methicillin-resistant *Staphylococcus aureus* (MRSA) endocarditis involving the free wall of the right atrium, with secondary seeding of the left knee. The patient was treated with intravenous antibiotics (vancomycin and piperacillin-tazobactam) and underwent two incision and drainage (I&D) procedures of the septic knee. Subsequently, his hemoglobin declined from 11.4 to 7.1 g/dL (reference range: 14.0–18.0 g/dL) for unknown reasons (Fig. 2). The patient’s blood typed as group A, D+, and multiple RBC units were transfused. Before the third I&D procedure, the patient received 3 units of fresh frozen plasma (FFP) for reversal of warfarin, which was being given for a thrombus that formed over the cardiac vegetation.

![Fig. 2](image-url)

**Fig. 2** Hematology values throughout the hospital course. (A) The WBC count peaked early in the hospital stay. The patient remained febrile throughout the hospitalization, however, developing disseminated infections involving the lungs, soft tissues, and bones. (B) The RBC count, including the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW), could not be reported for 24 days after the transfusion of FFP because of agglutination in the patient’s blood sample, refractory to 37°C warming. (C) Mild thrombocytosis developed into severe thrombocytopenia over 9 days after the transfusion of FFP. (D) The persistently declining hemoglobin was managed with numerous RBC transfusions throughout the hospitalization, several of which were with washed RBC units. Anti-M and anti-E were subsequently identified; anti-K could not be excluded. WBC = white blood cell; Tmax = maximum temperature; RBC = red blood cell; FFP = fresh frozen plasma; PLT = platelet; IVIG = intravenous immunoglobulin; DEX = dexamethasone; # = number of units transfused; Hgb = hemoglobin; Ab ID = antibody identification. *Anti-K could not be excluded.
Following the FFP transfusion, the RBC indices could not be reported because of RBC agglutination in the patient’s blood sample (Fig. 3), refractory to 37°C warming. Peripheral blood smears revealed large irregular clusters of RBCs, consistent with agglutination rather than rouleaux. No spherocytes or schistocytes were identified. After the FFP transfusion, his hemoglobin dropped from 8.1 to 5.6 g/dL. Upon retyping, the RBCs were 4+ reactive with anti-A reagent (Gamma-clone, ImmucorGamma, Norcross, GA) and weakly reactive with anti-B reagent (Gamma-clone, ImmucorGamma), representing a forward ABO type discrepancy. In addition, the patient’s sera was 3+ reactive with B cells (Referencells, ImmucorGamma) and weakly reactive with A_1 cells (Referencells, ImmucorGamma). The reactivity with reagent A_1 cells was not previously seen. The antibody screen was negative when using a 45-minute pre-warm method without polyethylene glycol enhancement.

To address the unexplained anemia, 8 units of RBCs were transfused over 5 days. Because the clinical team suspected bleeding rather than hemolysis, relevant laboratory values were only obtained at the beginning of this episode. These labs demonstrated a total bilirubin of 1.3 mg/dL (reference range: 0.3–1.2 mg/dL); lactic acid dehydrogenase (LDH) of 377 U/L (reference range: 120–246 U/L); and a haptoglobin of 177 mg/dL (reference range: 40–240 mg/dL), which was greater than 340 mg/dL prior to the FFP transfusion. A fecal occult blood test and urinalysis did not identify a source of bleeding. In addition, a computed tomography scan revealed only a small hematoma of the left thigh. The FFP transfusion also correlated with a rapidly declining PLT count, which was 472,000/mm^3 (reference range: 150,000–400,000/mm^3) prior to transfusion, and 8000/mm^3 9 days later. DIC was not favored as contributory to the thrombocytopenia because of the absence of schistocytes on peripheral blood films. Heparin was discontinued, even though the anti-platelet factor 4/heparin antibody test was negative. Perplexed by the hematologic complications, the clinical team consulted the blood bank.

The transfusion medicine service considered polyagglutination in the differential diagnosis. A DAT was performed and demonstrated 1+ mixed-field reactivity with polyspecific anti-human globulin (AHG), monoclonal anti-IgG, and anti-C3d. Next, a sample with a request for lectin panel testing was sent to a reference laboratory. Chloroquine-treated patient RBCs were weakly reactive with four sources of group AB plasma/sera at albumin (ALB)-37°C phase, including the diluent control, but were nonreactive at immediate spin (IS), room temperature incubation (RT), and ALB-IgG AHG phases. Patient plasma reacted with group A_1, A_2, and B reagent RBCs. In addition, the RBCs demonstrated reactivity with Glycine soja, but not Arachis hypogaea, Salvia horminum, or Salvia sclarea. This constellation of reactivity represents an extremely rare form of polyagglutination, not formally classified.

The blood bank informed the clinical team that plasma components should not be transfused and that units of RBCs and PLTs should be washed. Thereafter, the patient received multiple units of washed RBCs, although some units could not be washed because of staffing issues. The washed units of RBCs did not increase the hemoglobin by the expected increment. The patient developed anti-M and anti-E; anti-K could not be excluded (Fig. 2). All units of RBCs transfused during the hospitalization were AHG crossmatch-compatible. The severe thrombocytopenia was unresponsive to washed PLT transfusions. A course of intravenous dexamethasone resulted in concurrent resolution of the thrombocytopenia. After a complicated 44-day hospital course, the patient was transferred to a long-term care facility in stable condition. Since recovering from the infective endocarditis, the patient continues to see the neurology service at our hospital for treatment of his seizure disorder.

**Fig. 3** The peripheral blood film from the day after the transfusion of fresh frozen plasma showed large aggregates of erythrocytes not seen previously. Subsequent blood films showed progressively smaller aggregates and the development of severe thrombocytopenia. Blood films consistently showed neutrophils with toxic changes and few polychromatophilic macrocytes, but no spherocytes, schistocytes, or platelet aggregates were seen.
The purpose of this case study is to report on *Glycine soja* polyagglutination, a rarely described condition that has not been associated with infection. Polyagglutination is a rare condition that can be caused by cryptantigen exposure on RBCs through microbial enzyme activity. Because antibodies against cryptantigens are naturally occurring, transfusion of blood components, which contain plasma, can cause hematologic complications. The identification of polyagglutination involves lectin panel testing, which is available at reference laboratories but not available at most hospital transfusion services. Nevertheless, it is important to diagnose polyagglutination because patients with this condition are at risk for complications following the transfusion of plasma-containing blood components. Therefore, if possible, plasma components should be avoided, and units of RBCs and PLTs should be washed to avoid the passive transfer of antibodies targeting cryptantigens.

This report describes a 60-year-old African-American man who was found to have *Glycine soja* polyagglutination after developing a severe MRSA infection. Although the patient’s clinical course was complex, the patient’s history can best be explained by complications of polyagglutination. Prior to transfusion, the patient developed severe anemia of unknown etiology, possibly due to endogenous antibodies binding to the cryptantigens. The passive transfer of donor antibodies, targeting cryptantigens, can explain the onset of severe RBC agglutination, the forward ABO typing discrepancy, and the transfusion-refractory anemia. The reverse ABO typing discrepancy can best be explained by anti-M, which was first identified 3 weeks post-admission. The severe thrombocytopenia that rapidly developed after the FFP transfusion can be explained by cryptantigen exposure on the PLTs. Unfortunately, transfused units of washed RBCs and PLTs did not provide the increase in cell counts that was expected. This finding may be due to in vivo cryptantigen exposure on the transfused RBCs and PLTs.

Alternative diagnoses do not fully explain the patient’s complex hematologic findings. Post-transfusion purpura (PTP) can account for the severe thrombocytopenia (PLT <10,000/ mm³), as this occurred 9 days after the FFP transfusion. PTP is mediated by antibodies against class II HLA molecules or PLT-specific antigens, of which antibodies to human platelet antigen (HPA)-1a are most common. Alternatively, drug-induced thrombocytopenia, caused by vancomycin, could explain the severe thrombocytopenia. Studies to identify antibodies to HPA were not conducted, so PTP could not be formally excluded. However, neither PTP nor drug-induced thrombocytopenia is associated with RBC agglutination, anemia, or *Glycine soja* lectin reactivity. Drug-induced immune hemolytic anemia, due to piperacillin, can account for the positive DAT and anemia, although it cannot explain the severe thrombocytopenia or *Glycine soja* reactivity. A combination of drug-induced immune hemolytic anemia with PTP or drug-induced thrombocytopenia is unlikely, given that the onset of RBC agglutination and the rapid and severe decline in PLT count were temporally concurrent with the transfusion of FFP.

*Glycine soja* polyagglutination is distinct from other microbial-induced forms such as T activation (the most common form) or Th (an incomplete form of T activation), Tk, and Tx polyagglutination. Other unique features of *Glycine soja* polyagglutination include its association with *S. aureus*, RBC agglutination refractory to 37°C warming, and thrombocytopenia occurring in the absence of DIC.

Reports of *Glycine soja* polyagglutination have been described. A prospective study screened patients at high risk for polyagglutination with the soybean lectin *Glycine soja* and the peanut lectin *Arachis hypogaea*. Two out of 238 patients were identified with RBCs that reacted only with *Glycine soja*. One patient was a 71-year-old man with immunoblastic lymphoma. The other was a 52-year-old woman with a paravertebral mass. The authors included these two patients among a total of 18 with reported cryptantigen exposure. The study also examined 302 healthy adults in the control group and found no lectin reactivity among these patients. Another report of *Glycine soja* polyagglutination described a healthy blood donor from Bermuda whose RBCs demonstrated agglutination with the majority of recipient sera. Interestingly, the agglutination was refractory to 37°C warming. Further studies revealed that this form of polyagglutination was related to an inherited form known as Cad, which represents the strongest expression of Sd. The authors named this form Cad(Ber = Bermuda).

**Conclusions**

Polyagglutination is a rare, potentially fatal condition that may occur in septic patients, causing confounding anemia, thrombocytopenia, and DIC. These complications may occur in nontransfused patients but are more common in patients receiving plasma-containing blood components. Polyagglutination can be detected by demonstrating RBC agglutination with nearly all compatible adult sera, and lectin panel testing can confirm the suspected diagnosis. Transfusion protocols such as avoiding plasma components and the use...
of washed units of PLTs and RBCs are indicated to avoid potentially serious complications. Resolution of the underlying infection should lead to resolution of polyagglutination.

Acknowledgments

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References


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The blood group antigen Vel has been one of immunohematology’s greatest enigmas: the variation in antigen strength from one individual to another, the property of anti-Vel to readily hemolyze Vel+ red blood cells (RBCs), and the difficulty to screen for sufficient numbers of Vel− blood donors had made Vel a tough nut to crack. In 2013, a small, previously unknown protein called small integral membrane protein 1 (SMIM1) was identified on the RBC by three independent research groups using different approaches, and all three groups demonstrated that Vel− RBCs lacked SMIM1. This discovery correlated with homozygosity for deletion c.64_60del in SMIM1 and meant that for the first time there was a universal method to screen for Vel− blood donors. This finding was not the whole answer, however, and an explanation behind the variability in antigen strength was later shown to be due to polymorphism in SMIM1 intron 2, a region that is responsible for gene transcription. Clinically, anti-Vel is important and has caused severe transfusion reactions, although hemolytic disease of the fetus and newborn caused by anti-Vel is uncommon. However, while screening for Vel− blood donors has become easier, the function of SMIM1 is still unknown, and despite its well-conserved sequence across the animal kingdom, the enigma continues. *Immunohematology* 2017;33:56–59.

**Key Words:** Vel blood group, SMIM1, genotyping, blood group antigen

The first report of anti-Vel was published in the French journal *Revue d’Hematologie* by Sussman and Miller in 1952 and concerned a patient in New York who received 2 units of crossmatch-compatible blood.1 As reviewed by Sussman some 10 years later, the original proband was a patient with colon cancer who suffered a severe and immediate transfusion reaction following the second transfusion in a 3-day period. Although she had a history of uneventful pregnancies and transfusions prior to this occasion, her serum several days post-transfusion was strongly incompatible with the second unit transfused, although it remained compatible with the first unit.2 Subsequent screening of 10,000 donors with the patient’s serum in New York revealed only 4 compatible units in addition to the serendipitous compatible unit. This antibody displayed a clear dosage effect reacting with a titer of 64–128 with red blood cells (RBCs) from one group of individuals and more weakly (titer of 8–16) with RBCs from a second group that included the (presumed heterozygous) children of the patient.2

A second example of anti-Vel followed 3 years later, described by Levine et al., in a 67-year-old woman diagnosed with diverticulitis of the colon.3 She had received 1 unit of crossmatch-compatible blood 6 years previously following surgery for removal of an ovarian cyst, and she had a history of two pregnancies. At the time of the report, the patient’s serum contained a hemolytic antibody that was shown to be compatible only with the RBCs of the original proband and incompatible with over 1000 donors tested. Hemolytic reactivity has been a hallmark of anti-Vel, and Sussman had reported earlier that anti-Vel from 12 of 19 patients hemolyzed RBCs in vitro.2

In a study of Vel− families in northern Sweden, a disproportionate number of Vel− individuals were found to demonstrate a P2 phenotype,4 although this apparent association has been subsequently disproved.5 An association between Vel and Gerbich antigens was first demonstrated in a patient with anti-Ge.6 The patient’s RBCs were nonreactive with some Vel antibodies. Subsequent testing of 14 Vel antibodies with eight examples of Ge−2,−3,4 RBCs revealed three antibodies that failed to react with at least four of the RBC samples, and two other antibodies that were nonreactive with some. Subsequent elegant flow cytometry studies by Haer-Wigman et al.7 showed that there was no differential expression of glycoporphin C (GPC) on Vel− RBCs when compared with that on Vel+ RBCs, but that variation could be attributed to the rs1175550 single-nucleotide polymorphism known to influence Vel antigen expression (see the Molecular Basis section).

Lastly, serological evidence suggested that the high-prevalence antigen ABTI was related to Vel; in the original report of ABTI, it was noted that of eight Vel− samples, six reacted only weakly with anti-ABTI, and one did not react at all.8 In a second report, two new examples of anti-ABTI reacted only weakly with several examples of Vel− RBCs, confirming a serological connection, at least.9 Based on these reports, an International Society of Blood Transfusion (ISBT) collection, 200212, was created to accommodate Vel and ABTI. ABTI was returned to the 901 series (901015), following the identification of SMIM1 as the gene responsible for the Vel− phenotype. Indeed, SMIM1 sequence analysis of ABTI− individuals did not reveal a mutation that could account for the phenotype, and thus the ABTI blood group antigen remains uncharacterized.10
Although Vel has been detected on fetal RBCs from 12 weeks of gestation, it is expressed less strongly on cord cells than on adult cells.\textsuperscript{11,12} Vel is not detected on lymphocytes, granulocytes, or monocytes.\textsuperscript{13}

The nomenclature for Vel is presented in Table 1.

### Genetics/Inheritance

The Vel blood group antigen is inherited as a dominant trait. It is dependent on the expression of small integral membrane protein 1 (SMIM1), encoded by \textit{SMIM1}, which is located on chromosome 1 at 1p36. The prevalence of the Vel– phenotype in Europe varies from ~1 in 5000 in southern regions to ~1 in 1200 in Sweden.\textsuperscript{7,14,15} Its prevalence is much lower in people of African and Asian descent, where the prevalence of the mutated allele has been observed as 0.56 percent and 0.6 percent of these respective populations.\textsuperscript{7}

### Molecular Basis

Although Vel has been well defined serologically and there is a large set of observational data concerning the antibodies produced in response to Vel, nothing is known about the structure that carries Vel. The molecular basis of the Vel– phenotype was identified as a deletion of 17 bp in the coding region of the \textit{SMIM1} gene, \textit{SMIM1} c.64_80del.\textsuperscript{14,16,17} This deletion has been shown to be the primary molecular basis for the Vel– phenotype in all populations. Expression of Vel can be very weak, however, and varies considerably from the RBCs of one individual to another. Missense mutations at nucleotide position 152 (c.152T>A or c.152T>G; p.Met51Lys and p.Met51Arg, respectively) have been identified in individuals who type as Vel+,\textsuperscript{16,17} and expression studies in an HEK293T cell line using constructs with these two mutations demonstrated that Vel was markedly diminished in cells transfected with the \textit{SMIM1} c.152A construct and not expressed at all by HEK293T cells transfected with \textit{SMIM1} c.152G.\textsuperscript{7} Table 2 lists the molecular changes in the coding region of \textit{SMIM1} associated with the Vel antigen.

The identification of the molecular basis underlying the Vel– phenotype has enabled the development of polymerase chain reaction–based screening. This strategy has been successfully used to identify new Vel– donors in several laboratories.\textsuperscript{7,14,15,18}

There are other noncoding polymorphisms in the regulatory region in \textit{SMIM1} intron 2 that have also been shown to correlate with Vel antigen expression. The rs1175550 was first identified in 2012 in the investigation of genes that affect RBC morphology and function and was shown to be correlated with a lower mean corpuscle hemoglobin count (MCHC).\textsuperscript{19} This single nucleotide polymorphism has been extensively investigated, and the more common allele, rs1175550A, is associated with weak expression of Vel. The less frequent allele, rs1175550G, is associated with stronger expression that is zygosity-dependent.\textsuperscript{7,20} The mechanism for this is not entirely understood, although the change of A>G disrupts a recognition site for the erythroid-specific transcription factor, GATA-1. It was first postulated that this GATA-1 site acted as a negative regulator for \textit{SMIM1}, but further evaluation of this region has shown that there are other transcription factors that play a role—for example, TAL1, which was shown to bind preferentially to the rs1175550G allele and to upregulate Vel antigen expression.\textsuperscript{21,22} Another polymorphism, a trinucleotide insertion, rs143702418, also correlates with \textit{SMIM1} and with Vel antigen expression; the less frequent insertion is associated with downregulation and thus reduced expression.\textsuperscript{22}

### Biochemistry

\textit{SMIM1} encodes SMIM1. This protein is a single-pass membrane protein of 78 amino acids whose physiological role

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**Table 1. Nomenclature of Vel blood group system**

<table>
<thead>
<tr>
<th>Blood group system number</th>
<th>Blood group system</th>
<th>Antigen number</th>
<th>Alternate name</th>
<th>Prevalence</th>
<th>Gene</th>
<th>Chromosomal location</th>
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<td>Vel</td>
<td>VEL1</td>
<td>Vel</td>
<td>High</td>
<td>SMIM1</td>
<td>1p36</td>
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**Table 2. Alleles of the Vel blood group system recognized by the ISBT\textsuperscript{†}**

<table>
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<tr>
<th>Phenotype</th>
<th>Allele name</th>
<th>Nucleotide change in \textit{SMIM1}</th>
<th>Exon</th>
<th>Predicted amino acid change</th>
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<tr>
<td>Vel+</td>
<td>VEL*01</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vel−</td>
<td>VEL*−01</td>
<td>c.64_80delAGCCTAGGGGCTGTGTC</td>
<td>3</td>
<td>p.Ser22Glnfs</td>
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<tr>
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<td>VEL*01W.01</td>
<td>c.152T&gt;A</td>
<td>4</td>
<td>p.Met51Lys</td>
</tr>
<tr>
<td>Vel+\textsuperscript{w}/Vel−</td>
<td>VEL*01W.02</td>
<td>c.152T&gt;G</td>
<td>4</td>
<td>p.Met51Arg</td>
</tr>
</tbody>
</table>

\textsuperscript{†}ISBT = International Society of Blood Transfusion; \textsuperscript{w} = weak.

\textsuperscript{†}Only the polymorphisms in the coding region that have been found to affect antigen expression are shown. For further variation in the gene, see www.erythrogene.com and www.ensembl.org.
Antibodies in the System

Anti-Vel is the only antibody in the Vel blood group system. Antibodies to Vel are produced after immunization and are not naturally occurring. Unlike most antibodies to blood group antigens, anti-Vel sera are usually a mixture of IgM and IgG, fix complement readily in vitro, and demonstrate a wide thermal range of reactivity. Blood group antigen-specific IgM antibodies that do not readily class-switch to IgG antibodies are suggestive of an immune response to carbohydrate antigens such as A, B, H, P, and so forth. Unlike anti-Vel, however, these antibodies are almost invariably naturally occurring. Many Vel antibodies are readily adsorbed by rabbit RBCs or red cell stroma, although this is apparently not a specific adsorption but more that these reagents preferentially adsorb IgM antibodies. The ability to hemolyze Vel+ RBCs, at least in the days when serum was used for testing, has been a hallmark of anti-Vel and, in his review, Sussman reported that anti-Vel from 12 of 19 patients hemolyzed RBCs in vitro.

The serological reactivity of anti-Vel with RBCs treated with papain, ficin, trypsin, and α-chymotrypsin is greatly enhanced, and serum containing anti-Vel will often readily hemolyze papain-treated RBCs, for example. Vel antigen is also unaffected by neuraminidase treatment. The effect of 200 mmol/L dithiothreitol (DTT) treatment of Vel+ RBCs varies, with some anti-Vel recognizing a DTT-sensitive antigen, while the majority are unaffected. Rainer et al. demonstrated that of 11 antibodies tested, one sample was completely nonreactive with DTT-treated RBCs, four samples showed a decrease in reactivity of greater than or equal to 1+, and six samples were unaffected by the DTT treatment. Additionally, one report described the enhancement of anti-Vel reactivity following treatment of test RBCs with sodium hypochlorite.

Screening for Vel– blood donors has been hampered by the lack of suitable anti-Vel reagent, and blood centers have been restricted to limited volumes of the antibody from patients. Recently, a human monoclonal anti-Vel was produced by the French blood service, thus providing the potential for a long-awaited serologic screening and typing reagent. As of today, more than 950,000 blood donations in France have been screened for Vel with this monoclonal reagent, and 320 new Vel– blood donors were identified (unpublished data, personal communication with Michel Hennion, Etablissement Français du Sang Nord de France, Lille, France).

Clinical Significance

Anti-Vel is clinically important and has caused transfusion reactions ranging from mild to severe. In one case in which reactivity of an anti-Vel was attributed to a clinically insignificant cold-reactive antibody, 2 units of Vel+ blood were transfused, and the patient suffered a severe transfusion reaction and died 8 hours later. In France, it has been estimated that a mean of one severe hemolytic transfusion reaction caused by anti-Vel occurred per year between 1995 and 2009 (mean annual incidence of 1.7 per 10^8 inhabitants). Hemolytic disease of the fetus and newborn caused by anti-Vel has been described but is rare, possibly due in part to the weaker expression of Vel on cord RBCs. Examples of autoanti-Vel have also been reported. The first described was an IgM autoanti-Vel in an untransfused male patient with aplastic anemia. No shortened survival of Cr-labeled Vel+ RBCs was demonstrated. In contrast, a young girl with steroid-resistant warm autoimmune hemolytic anemia and an associated autoanti-Vel suffered a severe transfusion reaction when given 2 units of Vel+ blood. Vel– blood was tolerated well.

In conclusion, the Vel blood group system is a simple system consisting of just one antigen to date. Its complexity lies in the variation in antigen expression, which in turn makes anti-Vel sometimes difficult to identify, as well as the lack of availability of Vel– blood donors in most parts of the world. Vel is an antibody to be treated with respect because even weak antigen expression can have clinical consequences, as witnessed with the very first patient.
References

Rh is a complex blood group system with diverse genotypes that may encode weak and partial D variants. Standard serologic analysis may identify clinically significant D variants as D+; nevertheless, individuals with these D variants should be managed as D− patients to prevent antibody formation to absent D epitopes. Variant identification is necessary during pregnancy to allow for timely and appropriate Rh immune globulin (RhIG) prophylaxis for hemolytic disease of the fetus and newborn (HDFN) as D alloimmunization can occur with some D variants. Here, we describe two cases of the RHD*DAU5 allele associated with maternal alloanti-D in patients of African ancestry. Two obstetric patients were initially serologically classified as D+ with negative antibody detection tests on routine prenatal testing. Repeat testing at delivery identified anti-D in both patients with no history of RhIG administration or transfusion. DNA sequencing revealed that both patients possessed the RHD*DAU5 allele. Cord blood testing on both infants revealed positive direct antiglobulin test (DAT) results with anti-D eluted from the red blood cells (RBCs) of one of the infants. Despite the positive DAT, neither infant experienced anemia or hyperbilirubinemia. We document two cases of pregnant women whose RBCs expressed a partial D variant and were classified as D+ on the basis of standard serologic testing, resulting in subsequent failure to provide RhIG prophylaxis. Both cases were associated with alloanti-D formation but without significant HDFN. To our knowledge, these are the first reported cases of maternal alloanti-D associated with the RHD*DAU5 partial D variant. 

**Key Words:** Rh blood group system, partial D, DAU5, anti-D, alloimmunization

The Rh blood group system is the most complex and polymorphic blood group system; after ABO, it is the most clinically significant system in transfusion medicine. This complexity is primarily due to the Rh system being encoded by two homologous genes, RHD and RHCE, whose close proximity allows for conversion to occur between the two genes and the subsequent creation of polymorphic proteins that are responsible for the plethora of variants observed in the Rh system.1

The Rh system contains several highly immunogenic antigens. The D antigen in particular provokes antibody production in up to 80 percent of D− volunteers transfused with D+ blood.2 However, more recent retrospective analyses of D− patients who received D+ red blood cells (RBCs) have shown lower rates of antibody formation.3,4 D is also a significant cause of hemolytic disease of the fetus and newborn (HDFN), in which D− women with a D+ fetus may be alloimmunized during pregnancy or at delivery. Maternal alloimmunization and subsequent HDFN can be prevented in the vast majority of women with timely Rh immune globulin (RhIG) prophylaxis during pregnancy and after delivery. For this reason, the appropriate assignment of D antigen status is required for proper selection of blood products and perinatal management.

Although the majority of individuals can be correctly classified as D+ or D− using routine serologic methods, there are D variants that differ from wild-type D that create a gray area between these two categories. More than 460 RHD alleles have been identified with mutations that result in qualitative or quantitative changes in D expression.5 These variations in D are broadly, but somewhat artificially, categorized on the basis of serologic studies as weak D and partial D phenotypes.

Weak D variants involve changes in the D protein where at least one amino acid substitution occurs in the transmembrane or intracellular portions of the protein.6 By serologic definition, a weak D will give no or weak (≤2+) reactivity with immediate spin testing and will show stronger agglutination using an indirect antiglobulin test for detection. Prevalence of weak D varies by race and ethnicity, and current data suggest that individuals with the most common weak D types (types 1, 2, and 3), which make up the majority of weak D individuals found in Europeans,7 are not at risk of alloimmunization by D.8 These patients could safely receive D+ blood components and would not require RhIG prophylaxis. Unfortunately, the identification of patients with exceptional weak D types who are susceptible to D alloimmunization is not through serologic testing, but only through molecular analysis of the RHD gene.

The term “partial D” has been used to describe qualitative variants where amino acid substitutions in the extracellular portion of the D protein, or hybrid alleles, result in altered or missing epitope expression.6 This group can be very difficult to characterize serologically because of extensive variability in testing with different anti-D reagents. Consequently, RBCs with partial D variants may react strongly with initial
testing and be identified as D+, resulting in the individual not receiving RhIG prophylaxis during pregnancy or receiving D+ RBC transfusions. The frequency of anti-D formation occurring in partial D variants under these circumstances is not known. There are multiple case reports of obstetric patients with a partial D genotype who have formed anti-D as a complication of their pregnancy and have had infants demonstrating HDFN. Despite the potential for severe HDFN in these infants, the clinical manifestations have been mild in the majority of cases.

Partial D variants vary with race and ethnicity. Current serologic D typing strategies in North America and the UK are based on detecting partial D phenotypes more commonly found in white populations. For example, licensed anti-D reagents are required to react as D− in the setting of a partial DVI variant, which is the most commonly clinically significant partial D in this group. In black populations, the occurrence of partial D variants is more frequent, however, as is the frequency of anti-D in pregnancies with D+ mothers.

The DAU allele cluster has been described in individuals with African ethnicity, and several DAU variants have been shown to have variable reactivity when tested with common commercially available anti-D reagents. DAU is a phylogenetically related cluster of alleles with DAU0 postulated as the primordial allele. The DAU5 allele is defined by a mutation (F223V, E233Q, T379M) in RHD in exons 5 and 8, resulting in recombination of DAU0 and DVI. The DAU5 partial D allele has not been associated with anti-D resulting in HDFN through October 2016.

In this case report, we describe two obstetric patients whose RBCs with a partial D variant were classified as D+ during standard prenatal serologic testing resulting in subsequent failure to provide RhIG prophylaxis and subsequent development of alloanti-D at time of delivery but without HDFN.

Case Report

Patient A, a 34-year-old woman (gravis 6, para 2), who had emigrated from the Congo, had routine prenatal serologic testing at 13 weeks’ gestation completed at a perinatal testing laboratory. Testing for D included monoclonal blend Series 4 and Series 5 anti-D reagents (ImmucorGamma, Norcross, GA) on the Galileo Neo automated solid-phase testing platform (Immucor). Antibody screening was also performed on the Galileo Neo platform using Immucor 2 Cell Capture-R Ready Screen. All testing was completed in accordance with the manufacturer’s instructions. Patient A’s blood typed as group A, D+ (4+ reactivity with both Series 4 and Series 5 anti-D), and the antibody detection test was negative. No prior testing results were on record for this patient. The pregnancy was uneventful, with normal ultrasounds reported at 14, 21, and 32 weeks’ gestation.

The patient was admitted to the hospital at 40 weeks’ gestation in labor, and repeat serologic testing was performed at that time. In-hospital testing was performed on the Galileo Echo platform (Immucor) with Series 4 and 5 anti-D reagents. Her blood type was confirmed as group A, D+, with 4+ reactivity with both Series 4 and Series 5 anti-D reagents and 3+ reactivity with manual tube D testing with Series 5 anti-D. Her antibody detection test, also performed on the Galileo Echo using 2 Cell Capture-R Ready Screen (ImmucorGamma), was positive (3+ reactivity in both cells) with subsequent identification of the antibody as anti-D. The direct antiglobulin test (DAT) on the patient’s RBCs was negative. There was no record of RhIG administration or transfusion for the patient.

This specimen was tested at a reference laboratory with single nucleotide polymorphism (SNP) array analysis and subsequent DNA sequencing. Molecular analysis included genotyping/SNP analysis performed on the ID Core XT (Progenika-Grifols, Derio, Spain). Sanger sequencing was performed by use of the following: Progenika-Grifols (Medford, MA) using genomic DNA extracted from EDTA-whole blood, specific primers to PCR-amplify the 10 RHD exons and flanking introns, a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) to resolve the extension products by capillary electrophoresis, and SeqScape software (Applied Biosystems) to analyze data by comparison with the National Center for Biotechnology Information (NCBI) reference sequence. Genomic DNA sequencing detected the presence of the silenced RHD*Pseudogene and a partial D variant RHD*DAU5. The patient’s genotyping results on the ID CoreXT for the other Rh antigens predicted the RBCs to be C−, c+, E−, and e+, and a marker for r8 was not detected.

This patient delivered a healthy girl with APGAR scores of 7 and 9 at 1 and 5 minutes, respectively. In-hospital testing showed the infant’s cord blood type was group A, D+ with DAT reactivity of 1+. An eluate of the cord RBCs contained anti-D. The newborn’s plasma bilirubin levels over the next 2 days ranged from 102 to 151 μmol/L (normal: <200 μmol/L), thus prophylactic phototherapy was not required.

Patient B, a 31-year-old woman (gravis 3, para 1) originally from West Africa, had routine prenatal testing at 12 weeks’ gestation also at the same perinatal testing laboratory. Her blood type was group O, D+ (4+ reactivity with Series 4 and Series 5 anti-D), and her antibody detection test was negative,
consistent with historical records from previous testing in 2010. Her ultrasounds at 12 and 20 weeks' gestation were normal, and her pregnancy progressed without complication. Repeat serologic testing was performed in-hospital predelivery at 38 weeks’ gestation when the patient had a caesarian section for partial placental abruption. Her blood type was confirmed as group O, D+ (4+ reactivity with Series 4, 3+ with Series 5 anti-D). The patient’s antibody detection test was positive (3+ reactivity in two screening cells) with antibody identification confirming anti-D. DAT was negative. There was no record of RhIG administration or blood transfusion for the patient. This maternal specimen was also forwarded for molecular analysis. Genomic DNA sequencing detected the presence of a hybrid RHD*IIIa-CE(4-7)-D and a partial RHD*DAU5 allele. The patient’s genotyping results on the ID CoreXT for the other Rh antigens predicted her RBCs to be C+, E−, c+, with the hybrid RHD*IIIa-CE(4-7)-D encoding variant (partial) C antigen expression. The patient’s phenotype was confirmed as C+ serologically.

The patient delivered a healthy boy with APGAR scores of 9, 10, and 10 at 1, 5, and 10 minutes, respectively. Cord specimen testing showed blood group O, D+. DAT was positive with weak reactivity, and a subsequent eluate performed on cord RBCs was negative. The infant had a transcutaneous bilirubin of 147 μmol/L after 24 hours and was discharged home without prophylactic phototherapy.

Discussion

Here, we have described two cases of the partial D variant DAU5 associated with maternal anti-D in unrelated African patients. One mother’s RHD genotype was RHD*Pseudogene/RHD*DAU5 and the RHD genotype of the other mother was RHD*DAU5/RHD*IIIa-CE(4-7)-D. In both cases, the patients were classified as D+ on the basis of concordant strong reactivity with two different anti-D reagents. As a result, neither mother received RhIG, and both were subsequently alloimmunized for D during their pregnancies.

These cases emphasize the importance of being able to recognize and identify weak and partial D phenotypes that place women of child-bearing potential at risk of forming alloanti-D so that subsequent RhIG prophylaxis can be provided as well as avoiding transfusion of D+ RBCs.

To avoid this complication, it has been recommended to test samples from obstetric patients and potential transfusion recipients with two specifically selected monoclonal anti-D reagents, that have dissimilar specificities, in an attempt to increase the likelihood of identifying variant D expression and to potentially identify those in which genotyping may be useful. The cases we describe did not exhibit the serologic discrepancies in D typing that would suggest the presence of a D variant, a phenomenon that has been reported previously. The epitopes altered by the mutation remained fully serologically reactive with standard commercial reagents used on both automated and manual testing platforms. These cases may represent variations in the particular source and specificity of antisera, resulting in failure to detect this variant antigen.

Fortunately, neither infant in the reported cases experienced significant hyperbilirubinemia. The infants did not develop anemia nor require phototherapy, and both were discharged from the hospital without an extended stay.

Obstetric patients with a partial D phenotype are known to have the potential to form anti-D during pregnancy, and their infants are at risk for HDFN. Despite the potential for severe HDFN due to anti-D, the clinical manifestations in patients with partial D have been mild in the majority of cases except for those in mothers with DVI variants.

The reason for partial D variants to be associated with a lesser degree of morbidity when compared with HDFN in D− individuals with alloanti-D is unknown. One hypothesis proposes that because D variants do express numerous epitopes of the D antigen, the associated anti-D may not have a broad specificity and hence has diminished hemolytic potential compared with anti-D in a D− individual.

These two cases highlight the limitations of routine serologic testing to detect some partial D variants. A commentary by Sandler et al. of the AABB–College of American Pathologists (CAP) Working Group proposed selective integration of RHD genotyping in routine obstetric and pre-transfusion testing to improve the accuracy of D typing results, to reduce unnecessary RhIG administration in women with serologic weak D phenotypes, and to overall decrease transfusion of scarce D− RBCs to patients with weak D phenotypes. The AABB–CAP Working Group did not address management of partial D phenotypes, except in the management of discordant D typing results, in which case they recommend RHD genotyping. The incorporation of genotyping into prenatal testing may offer a solution, although the significant cost of this methodology limits use in routine testing. The implementation of genotyping of D+ women of childbearing age who are of African descent may offer a more feasible solution to this issue.

To our knowledge, these are the first reported cases of maternal alloanti-D associated with the RHD*DAU5 partial D variant. Neither infant experienced clinically significant
Maternal alloanti-D and the DAU5 allele

anemia nor hyperbilirubinemia although the RBCs of both infants reacted in the DAT.

References


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Attention: SBB and BB Students

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The presence of the FORS1 antigen on red blood cells was discovered relatively recently, and in 2012, the International Society of Blood Transfusion (ISBT) acknowledged FORS as blood group system number 031. This rare antigen is carried by a glycosphingolipid and formed by elongation of the P antigen. Most people have naturally occurring anti-FORS1 in their plasma. The clinical significance of these antibodies is unknown in the transfusion setting, but they can hemolyze FORS1+ erythrocytes in the presence of complement in vitro. First believed to be part of the ABO system, it was later shown that the gene encoding the glycosyltransferase giving rise to FORS1 expression is GBGT1. This gene had previously been deemed nonfunctional in humans, but a mutation, so far only detected in FORS1+ individuals, restores the enzymatic activity. Tissue distribution of the antigen in FORS1+ individuals has not been studied in detail, although the gene is expressed in several cell types. The antigen itself is known to be a receptor for various pathogens and toxins and has been detected in different forms of cancer, but the implications thereof are not fully understood. Immunohematology 2017;33:64–72.

Key Words: FORS, FORS1, low-prevalence antigen, Forssman, A<sub>pu</sub>

The first blood group system was discovered in 1900 by Landsteiner,<sup>1,2</sup> and its antigens were later characterized as glycans.<sup>3,4</sup> Since then, ABO has been joined by six other systems that are also of carbohydrate nature: P1PK, H, Lewis, I, GLOB, and FORS (www.isbt-web.org). The defining antigens in these blood group systems are all determined by immunodominant sugar moieties of glycoproteins and/or glycosphingolipids. The function of these structures is unknown, but it has been suggested that they are a part of our innate immune defense.<sup>5</sup> Carbohydrate expression is relatively well conserved in mammals, but the variation seen both within and between species may reflect evolutionary developments to differentiate the individual species-specific susceptibility to various pathogens.<sup>5</sup> Another common principle for these blood group systems is that naturally occurring antibodies are formed against the carbohydrate antigens that are lacking. These antibodies have the potential to neutralize pathogens expressing the corresponding glycan epitopes.<sup>7,8</sup> In modern medicine, however, they mainly constitute a significant transfusion and transplantation barrier and can also cause fetomaternal incompatibility. This review will summarize the current knowledge on the newest of our carbohydrate blood group systems, FORS, acknowledged by the International Society of Blood Transfusion (ISBT) in 2012. For obvious reasons, the body of knowledge is still limited, especially when it comes to the clinical significance of this system.

Forssman: Historical Aspects

The first mention of what was later designated the Forssman (Fs) antigen was reported in 1907 by Frouin.<sup>9</sup> This heterophilic antigen was re-identified in 1911 by John Forssman (1868–1947), professor of microbiology, pathology, and general medicine at Lund University, Lund, Sweden (Fig. 1). He immunized rabbits with tissue from guinea pig or horse, and these rabbits produced an antibody that was shown to hemolyze red blood cells (RBCs) from sheep in the presence of complement. Tissue from other species (e.g., cow or rat) did not induce the same immune response.<sup>10</sup> In honor of his detailed description of these experiments, the antigen recognized by these antisera was named after Dr. Forssman.

By using the antibodies towards this (then structurally undefined) antigen, different animal species were categorized to be either Fs+ or Fs–.<sup>11</sup> Humans were defined as an Fs– species, although there are reports claiming the presence of this structure in human tissue from malignant tumors<sup>12–17</sup> and even a few publications on its presence in normal tissue.<sup>12,18</sup>
The Fs antigen is a carbohydrate moiety present in various tissues, including RBCs, depending on species. The structure of the antigen was resolved some 60 years after Dr. Forssman’s experiments and identified to be GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer by Siddiqui and Hakomori.\cite{Siddiqui1987} A few years later, the structure was confirmed by mass spectrometry.\cite{Forsman1989}

Over the years, the naturally occurring antibodies towards Fs present in the vast majority of people have been associated with different autoimmune diseases such as rheumatoid arthritis,\cite{Anderson2001} Guillain-Barré syndrome,\cite{Guillain1951} and Graves’ disease.\cite{Graves1926} The significance of these findings is still unclear. In recent years, anti-Fs has been reported to play a role in the xenotransplantation setting.\cite{Stamps2001, Sheehy2005, Tyan2008}

### Nomenclature

In light of recent developments, a motion was made to make FORS the 31st blood group system, and this proposal was accepted by the ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology at its meeting in 2012.\cite{ISBT2012} FORS1 (031001) is so far the only antigen of the FORS blood group system. The correct nomenclature is shown in Table 1.

<table>
<thead>
<tr>
<th>System name</th>
<th>FORS (ISBT no. 031)</th>
</tr>
</thead>
<tbody>
<tr>
<td>System symbol</td>
<td>FORS</td>
</tr>
<tr>
<td>Antigen name</td>
<td>FORS1 (ISBT no. 031001)</td>
</tr>
<tr>
<td>Carrier molecule</td>
<td>Forsman glycosphingolipid</td>
</tr>
<tr>
<td>Synthesized by</td>
<td>Forsman synthase</td>
</tr>
<tr>
<td>Gene name</td>
<td>GBGT1</td>
</tr>
</tbody>
</table>

ISBT = International Society of Blood Transfusion.

### Transition from ABO to FORS

The history of the human FORS1 antigen on RBCs originates back to 1987 when Dr. Robert Stamps and co-workers reported what appeared to be a peculiar weak A subgroup that they termed A_x. This phenotype was present in healthy individuals from three different unrelated English families.\cite{Stamps1987} The “p” in A_x stands for the reaction observed with Helix pomatia, and “ae” identifies its ability to adsorb and elute some polyclonal anti-A reagents. For a long time, the antigen underlying this phenotype was considered to be one of the many Aweak variants within the ABO blood group system (ISBT no. 001) and, as stated in a textbook (“Had the authors chosen to call A_x a form of A, it is doubtful that many would have argued”), it was considered very similar to one of the less uncommon ABO subgroup phenotypes, A_x.\cite{Salama2017}

Genomic typing of ABO was performed in our laboratory on samples from two unrelated individuals displaying the A_x phenotype. The purpose was to characterize the underlying A allele that caused the hypothesized weak expression of A. The surprising result was that both individuals were homozygous for the common deletion, c.261delG, in the most frequent O alleles (ABO*0.01.01/*0.01.01 and ABO*0.01.01/*0.01.02) and should clearly phenotype as group O. Based on this result and in collaboration with Professor Steve Henry in Auckland, New Zealand, and scientists at the University of Gothenburg in Sweden, we subsequently came up with the hypothesis that the A_x phenotype must be due to an A-like but ABO-independent antigen. In 2013, we published the biochemical and genetic characterization of the new blood group.\cite{Henry2013} We showed that the gene that gives rise to the A_x phenotype was indeed not ABO but GBGT1 (globoside α-N-acetylgalactosaminyltransferase, EC 2.4.1.88), the Forssman gene. Based on the data from that report, which included extensive serologic testing, structural analysis of the glycolipid antigen, and transfection studies, all the data were in place to promote A_x to become the new blood group system FORS. The phenotype name A_x was deemed obsolete, and the antigen was accordingly named FORS1.

### Biochemistry

FORS1 is a glycosphingolipid that is part of the globo-series synthesis pathway. This pathway also harbors other carbohydrate blood group antigens such as P^e, P, NOR, LKE, and the globo version (type 4) of H, A, and B. Depending on the sugar moiety added to lactosylceramide, different glycosphingolipid pathways are created. The globo-series is initiated by addition of a galactose (Gal) in the α1-4 position to lactosylceramide, and this structure constitutes the P^e antigen (also known as globotriaosylceramide, Gb3, or CD77). Adding an N-acetyl-d-galactosamine (GalNAc) in a β1-3 position to the P^e structure will result in the P antigen (globoside, Gb4), and this structure is the precursor for the enzyme that makes FORS1, the Fs synthase (Fig. 2). It has been suggested that the Fs antigen may also occur as the glycan portion of glycoproteins, but this has not yet been corroborated in humans.\cite{Kobayashi2000, Kato2001}

The Fs synthase has a topology that matches well with the structure of a single-pass type II transmembrane glycosyltransferase. In analogy with the blood group A transferase, 3-α-N-acetylgalactosaminyltransferase (GTA), the Fs synthase transfers a terminal GalNAc in α1-3 linkage.
Instead of the H antigen, however, the required precursor for Fs synthase is the P antigen (globoside, Gb4), as mentioned earlier (Fig. 3).

Based on sequence similarities, glycosyltransferases have been categorized into families in the Carbohydrate-Active EnZymes (CAZy) database. The Fs synthase is encoded by the GBGT1 gene and belongs to the GlycosylTransferase 6 (GT6) family alongside, for example, ABO (Table 2).

Until the discovery of FORS1+ individuals, GTA and 3-α-galactosyltransferase (GTB)—responsible for blood group A and B expression, respectively—were considered to be the only functional glycosyltransferases in the human GT6 family. The other members (Table 2) had been deemed nonfunctional. The number of GT6 genes varies from species to species, and even within phylogenetic groups, the type and number of genes can vary significantly.

### Genetic and Molecular Basis

The sequence homology between the members of the GT6 family indicates that these genes were derived from the same ancestral gene where gene duplications have occurred with subsequent divergence. The expansion and deletions of GT6 genes during vertebrate evolution suggests that this multigene family follows a birth-and-death evolution type.

### Table 2. Details about the most investigated members of the human GlycosylTransferase 6 (GT6) gene family

<table>
<thead>
<tr>
<th>EC no.</th>
<th>Glycosyltransferase</th>
<th>Donor</th>
<th>Acceptor</th>
<th>Gene</th>
<th>Chromosome location</th>
<th>Transcribed</th>
<th>Translated</th>
<th>Produced antigen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.1.37</td>
<td>3-α-galactosyltransferase</td>
<td>UDP-Gal</td>
<td>H antigen</td>
<td>ABO</td>
<td>9q34</td>
<td>Yes</td>
<td>Yes</td>
<td>B</td>
<td>33</td>
</tr>
<tr>
<td>2.4.1.40</td>
<td>3-α-N-acetylgalactosaminyltransferase</td>
<td>UDP-GalNAc</td>
<td>H antigen</td>
<td>ABO</td>
<td>9q34</td>
<td>Yes</td>
<td>Yes</td>
<td>A</td>
<td>33</td>
</tr>
<tr>
<td>2.4.1.87</td>
<td>3-α-galactosyltransferase</td>
<td>UDP-Gal</td>
<td>Galβ1-4GlcNAc-R</td>
<td>GGTA1</td>
<td>9q33</td>
<td>Yes</td>
<td>No</td>
<td>α-Gal epitope</td>
<td>34, 35</td>
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<tr>
<td>2.4.1.87</td>
<td>3-α-galactosyltransferase</td>
<td>UDP-Gal</td>
<td>Galβ1-4Glcβ1-Cer</td>
<td>A3GALT2</td>
<td>1p35</td>
<td>Yes</td>
<td>No</td>
<td>iGb3</td>
<td>36</td>
</tr>
<tr>
<td>2.4.1.88</td>
<td>globoside</td>
<td>UDP-GalNAc</td>
<td>Globoside/P antigen/Gb4</td>
<td>GBGT1</td>
<td>9q34</td>
<td>Yes</td>
<td>No/Yes</td>
<td>Forssman</td>
<td>30, 37, 38</td>
</tr>
</tbody>
</table>

EC no. = enzyme commission number.
The cDNA corresponding to the canine Fs synthase was cloned in 1996 by Haslam and Baenziger. A 347–amino acid (aa) open reading frame (ORF) was predicted, and as previously mentioned, the overall structure matches well with a type II transmembrane glycosyltransferase. The canine Fs synthase construct was tested in a transfection experiment where it was shown to result in expression of the Fs antigen in the COS-1 cell line.

The human GBGT1 equivalent was cloned in 1999 by Xu et al. It is situated on chromosome 9 (9q34) in close proximity to the ABO gene and consists of seven exons spanning over ~11 kb (Fig. 4). The human aa sequence showed a 45 percent homology to GTA and GTB. The ORF (1041 nucleotides [nts]) encodes a 347-aa-long protein with a molecular weight of ~40 kDa. In that study, the authors also showed by transfection studies that the human Fs synthase did not possess the ability to synthesize Fs glycolipid, as the canine version did. This finding corroborated the known expression of Fs in dogs and its absence in humans. Chimeric constructs, namely, combinations of canine and human sequences, showed that the human catalytic site was inactive, but no precise reason as to why was proposed. RNA expression in a variety of tissues was measured, and transcripts were detected in all tissues examined but did not appear to give rise to Fs antigen expression. The authors hypothesized that, given the high expression of RNA, this human Fs synthase might have acquired an altered enzyme specificity. Others have argued that the high homology with canine Fs synthase and the presence of transcripts suggest that the human ability to synthesize Fs was lost quite recently in an evolutionary perspective.

Svensson et al. reported the first verified and structurally confirmed expression of the Fs antigen on human RBCs. In addition to extensive serological and biochemical verification of the antigen, it was shown that individuals with the Apar phenotype had a single nucleotide polymorphism (SNP) in the ORF of the GBGT1 gene. The mutation c.887G>A gave rise to an aa change, p.Arg296Gln, and we hypothesized that this was the key residue for the gain of function for the Fs synthase in these individuals. This idea was supported by genetic analysis of available members in the family pedigrees and also confirmed by transfection of Fs– cell lines that became Fs+ when 887A but not 887G Fs synthase-encoding constructs were introduced. The same finding, but from a different angle, was simultaneously corroborated in a study by Yamamoto et al., in which they showed aa position 296 to be essential for Fs antigen expression. This was hypothesized by comparing aa sequences of Fs– and Fs+ species, thereby identifying candidate aa positions in the enzyme conserved in Fs+ species and differing from Fs– species. This approach revealed three positions for further testing: c.536C>T (p.Ile179Thr), c.688G>A (p.Gly230Ser), and c.887A>G (p.Gln296Arg). Each of these substitutions was evaluated by introducing them into a mouse Fs construct (an Fs+ species) for subsequent expression in the COS-1 cell line. Two positions were shown to be crucial for Fs antigen expression, when changing nts c.688 and c.887 to the murine equivalent in a human construct, the Fs synthase activity was completely restored to allow synthesis of Fs antigen in the COS-1 cell line. The conclusion drawn was that p.Ser230Gly and p.Gln296Arg constitute the cause of Fs negativity in humans, although the study by Svensson et al. showed that p.Arg296Gln suffices for RBC expression of Fs activity.

The prevalence of c.887G>A is very low, less than 1 percent in the populations tested, but c.887G>A has been detected on two distinct alleles in different families. In addition to the investigated pedigrees from Stamps et al., the mutation has also been found in a database search in the NHLBI-ESP (National Heart, Lung, and Blood Institute–Exome Sequencing Project), although the phenotype has not been confirmed there. A mutation, c.363C>T, that gives rise to an early stop codon in exon 7 truncates the Fs synthase prematurely and is predicted to cause lack of the enzymatic site. This null allele is fairly common and shows a prevalence of greater than 4 percent according to the Erythrogene database (www.erythrogene.com), an open Web resource that summarizes the allelic variation in all blood group genes in the 2504 individuals (from 26 different populations around the globe) included in the 1000 Genomes Project. Multiple other GBGT1 variants were observed in this database but, for most, their prevalence is very low. Among the 68 GBGT1 alleles noted in Erythrogene so far, only 4 have an allele frequency greater than 1 percent, and 32 were found only once and may need

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**Fig. 4** Genomic organization of the GBGT1 gene. The seven exons are represented by numbered rectangles drawn to scale (introns, represented by a gray line, are not). Black vertical lines in exons 2 and 7 indicate the start and stop codons, respectively, and the open reading frame is shown in dark gray.
confirmation. In fact, the two alleles that give rise to FORS1 were not found at all in the 1000 Genomes samples but are included only in the ISBT reference lists (www.isbt-web.org).

**Antigens and Antibodies in the System**

**Antigens**

Because FORS1+ is very rare, its real prevalence is unknown, but so far only three families with the A_{par} phenotype (now referred to as FORS1+) have been reported. In addition, a single individual with the same genetic variant has been noted, but no phenotypic data were available. So far, the antigen has only been detected in individuals of European extraction. The Fs glycosphingolipid structure is normally not expressed on any type of human tissue and is widely regarded as a structure only found in certain animal species. Because screening for FORS1 is not a standard procedure, it is unlikely to detect this rare phenotype on a routine basis. Nevertheless, if detection of c.887G>A were to be integrated on commercially available genotyping platforms in the future, screening could easily become part of daily practice. A possible reason to do this would be to rid blood banks of donated RBC units expressing FORS1. This may become an issue of debate if the naturally occurring antibodies against FORS1 (discussed in the next section) found in the plasma of most people were found to be clinically significant—that is, hemolytic in vivo.

In the original report by Stamps et al., the A_{par} phenotype was detected by a cross-reaction of a polyclonal anti-A, but because polyclonal ABO reagents are scarce today and standard ABO phenotyping is now performed with monoclonal reagents, detecting this rare phenotype by ABO typing is unlikely. In a study by Barr et al., the authors tested 19 monoclonal anti-A reagents against kodecytes, RBCs uploaded with a FORS1 pentasaccharide Function-Spacer-Lipid (FSL) construct, and did not notice any of the cross-reactivity seen with polyclonal reagents.

**Antibodies**

In analogy with ABO, most humans have naturally occurring antibodies to the FORS1 antigen they lack. These antibodies are believed to be formed in response to microbial surface glycan structures in the commensal gut flora, because some of the latter are very similar to carbohydrate blood group antigens. Their presence may be a microbial strategy to evade the immune defense in the host in accordance with the molecular mimicry concept. The human anti-Fs are mainly of IgM type, but there may also be a component of IgG. Svensson et al. reported crossmatch reactivity with FORS1+ RBCs to be about 6 percent by the indirect antiglobulin test, and approximately 23 percent caused direct agglutination. Great variation in antibody strength was seen among the almost 300 samples of random blood donor plasmas tested.

In a recent screening study, which included 800 blood donors, it was shown that all plasma samples but one had antibodies against sheep RBCs, which are known to express very high levels of the Forssman antigen. These donor plasma samples also reacted with kodecytes. The possible influence of other human anti-sheep xenoantibodies as reported by Strokan et al. and potential cross-reactions of the polyclonal anti-A present in human plasma were not discussed in that report.

The clinical relevance of anti-FORS1 in a transfusion setting remains unclear, but it has been shown that human ABO-compatible plasma has the ability to hemolyze FORS1+ RBCs in the presence of complement. This finding is further emphasized when RBCs have been papainized. From a transfusion point of view, FORS1+ units should only be transfused following a negative crossmatch until we know more (i.e., electronic crossmatch using type-and-screen should not be used). The electronic crossmatch is currently used in many parts of the world; thus, a FORS1+ unit may be 4+ incompatible and still be transfused. Because of the low prevalence of this phenotype, it is still questionable whether genetic screening to exclude FORS1+ donors is worthwhile.

**Tissue Distribution**

The heterophilic Fs antigen has been recognized in several species, both mammals and others. Because it is expressed in some species, but not in others, the presence or absence of the Fs antigen has classified species as Fs+ or Fs−. Sheep, dog, cat, and mouse are examples of Fs+ animals, whereas pigeon, rabbit, cow, and rat do not express the Fs antigen. The level of antigen seen on RBCs varies; some are high expressers, like sheep RBCs, with lower expression seen on RBCs from dogs and cats. There is also heterogeneity with respect to what cell types carry the antigen; in many species, Fs antigen expression is not found on RBCs but is restricted to other tissue. Purification of the Fs glycolipid from different species has been performed from kidney tissue and intestinal mucosa, among others. Humans and apes have been considered Fs−, but there are some reports of Fs glycolipid expression in normal human non-hematopoietic tissue. Because the Fs synthase has been deemed nonfunctional in the majority of humans, the mechanism underlying this expression remains unknown.
Although the expression of Fs antigen on human RBCs was unequivocally shown by Svensson et al., the presence of the antigen in non-erythroid tissue in these individuals was not investigated. It has been shown that the GBGT1 gene is transcribed in different human tissues, but this finding is not expected to give rise to any Fs antigen expression in FORS1–individuals. It is tantalizing, therefore, to speculate that an individual expressing the antigen on RBCs may express the antigen in other tissues as well, since other antigens in the globo-series like P and Pk are expressed, for example, in the urinary tract. The mRNA levels as measured in blood did not show any significant difference between FORS1+ individuals and FORS1–controls, but the c.887G>A-activated version of the GBGT1 gene is able to give rise to significant levels of FORS1 on RBCs. This finding begs the question in these FORS1+ individuals: Is the Fs glycosphingolipid expressed in all the various tissues where the gene is transcribed? Post-transcriptional and epigenetic factors may of course influence this, and so does the availability of acceptor substrate for the translated enzyme. Another major question that remains to be answered is how is this gene regulated.

**Disease Associations**

**Infections**

The first type of structures that many pathogens encounter in the host are glycans on cell surfaces and in mucus. There are numerous examples of bacteria, viruses, and parasites that invade/impact the host cells via these carbohydrate structures, some of them being blood group antigens. For example, Helicobacter pylori, known to cause gastritis, uses the Leα antigen, and the norovirus that causes gastroenteritis is dependent on the ABO secretor status of the host. In the most virulent species of the malaria parasite, Plasmodium falciparum, it has been shown that the parasite-rosetting ligands PIEMP1 and RIFINs bind to RBC surface structures including CR1 and A antigens, respectively. For instance, when infected RBCs form rosettes with uninfected RBCs in a group A individual, the rosettes are bigger and not as easily disrupted as the ones formed in group O individuals. In fact, the latter interaction may be the reason why blood group O protects against severe outcomes from cerebral malaria. Currently, however, there is no human disease that shows a clear association with the FORS1+ phenotype, possibly because such individuals have only recently been identified.

The expression of different carbohydrate structures may determine host tropism to microorganisms and, as with many other antigens, the Fs structure can act as an involuntary receptor for microbes. Xu et al. used a canine GBGT1 construct for transfection experiments and expressed the Fs antigen in either a human or a primate cell line. Following transfection, canine uropathogenic Escherichia coli were able to bind the Fs-expressing cells but not the wild-type mock-transfected cells.

P-fimbriated E. coli strains may express papG or prsG adhesins, which recognize blood group structures. Whereas papG prefers the Galα4Gal motif in glycolipids like P1 and Pk antigens, the prsG adhesion binds to the Fs-terminating structure GalNAcα3GalNAc. It has also been shown that prsG+ E. coli can agglutinate RBCs from FORS1+ individuals and from sheep but not control RBCs from healthy blood donors, independent of ABO group. The clinical consequence of this is not clear but proposes a potential for trans-species infections to occur if the Fs antigen is present on urinary tract epithelium, as many of the related glycolipids are.

Expressing the Fs antigen can also protect the host in some scenarios. The susceptibility to Shiga toxin (Stx) 1 is lowered because the preferred binding site (the P antigen, Gb4) is converted into the Fs antigen. On the other hand, Stx2e isolates from pigs and humans showed a clear interaction with the Fs glycosphingolipid in another study, whereas Stx1 and Stx2 did not.

**Cancer**

The complex role of glycosylation in cancer is widely acknowledged but not fully understood. Glycosylation alterations such as over- and underexpression and aberrant expression is associated with oncogenic transformation. The COSMIC (Catalogue of Somatic Mutations in Cancer) database lists 66 mutations found in the GBGT1 gene in relation to various forms of malignancies. There are a number of reports of the Fs antigen being expressed in different forms of cancer and malignant tumor tissue, but the mechanism behind this expression is still to be elucidated. Interestingly, Hakomori et al. reported that when the Fs glycolipid was detected in malignant tissue, the surrounding normal tissue typed Fs--; conversely, when present in normal tissue, the Fs glycolipid was not detectable in tumor tissue. The authors also suggested that the incompatible A-like antigen seen in some tumors in non-A individuals may instead be the Fs antigen.

The mechanisms behind this enigmatic expression of the Fs antigen are yet to be resolved, and there are many aspects to take into consideration. In an interesting study from 2017, Yamamoto et al. showed that other glycosyltransferases can be pushed to synthesize the Fs antigen in the laboratory setting based on artificial (not naturally occurring) recombinant...
glycosyltransferases. In a situation like cancer, where genetic events are not as closely surveilled and corrected, mechanisms like this may interact with other factors that need to be further examined, such as aberrations in splicing, post-translational modification of the protein, various epigenetic factors, and so forth. As an example of this, Jacob et al. showed that GGBP1 expression in ovarian cancer cells could be epigenetically downregulated through hypermethylation of the promoter, and this finding was also correlated to protein, Fs synthase, expression.

Summary

The FORS blood group system is young in the context of transfusion medicine. Nevertheless, its single antigen, FORS1, and its corresponding antibody specificity have played a role in medicine for more than a century. The presence and significance of FORS1 in non-erythroid human tissue is still under debate, both in health and disease. The rare FORS1+ RBC phenotype is important to bear in mind when running into unexpected results with lectins and in ABO discrepancies, especially because most people have naturally occurring anti-FORS1 in their plasma. Solving the biochemical and genetic basis underlying human FORS1 expression has added valuable information to our knowledge base about carbohydrate blood group antigens.

Acknowledgments

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References


For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, contact Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at Sandra.Nance@redcross.org.

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A suspected delayed hemolytic transfusion reaction mediated by anti-Jo


A 32-year-old African-American woman with a history of sickle cell disease presented for surgical evaluation of left total hip arthroplasty due to avascular necrosis of the femoral head. In anticipation of a complex orthopedic procedure, pre-surgical blood work was ordered. The patient’s Fenwal blood sample typed as group O, D+. Although the patient had a history of anti-Fy\(^a\), the antibody identification was inconclusive, so the workup was sent to a reference laboratory. The patient was last transfused with red blood cells (RBCs) 2 years earlier, but had no history of transfusion reactions. Due to surgery, the patient’s hemoglobin (Hb) decreased from 10.2 g/dL (preoperative) to 8.6 g/dL (postoperative). One unit of weakly crossmatch-incompatible Fy\((a–)\), C–, E–, K–, and sickle cell hemoglobin S (HbS)-negative RBCs was transfused without incident, and the patient was discharged. Several days later, the reference lab reported two new specificities, anti-Jo\(^a\) and anti-Jk\(^b\). Fortunately, the transfused RBC unit was Jk\((b–)\). Therefore, the crossmatch incompatibility was attributed to anti-Jo\(^a\), which targets a high-prevalence antigen found in 100 percent of most populations. Two weeks after discharge, the patient returned in sickle vaso-occlusive pain crisis. The patient was clinically stable, but her Hb was 6.7 g/dL. One unit of Fy\((a–)\), Jk\((b–)\), C–, E–, K–, HbS– RBCs, which was weakly crossmatch-incompatible, was transfused. The following day, her Hb was unchanged, lactic acid dehydrogenase increased from 951 to 2464 U/L, potassium increased from 3.7 to 4.6 mEq/L, creatinine increased from 0.60 to 0.98 mg/dL, and the patient developed a 38.4°C fever. These findings are consistent with a delayed hemolytic transfusion reaction (DHTTR), mediated by anti-Jo\(^a\), occurring 2 weeks after the first RBC transfusion. Further care could not be provided because the patient left the hospital against medical advice. The purpose of this case study was to report findings consistent with a DHTR attributed to anti-Jo\(^a\), an antibody with relatively unknown clinical significance.

**Immunohematology** 2017;33:73–75.

**Key Words:** Jo\(^a\), delayed hemolytic transfusion reaction (DHTTR), Dombrock blood group system, high-prevalence antigen

The Dombrock blood group system consists of antithetical antigens, Do\(^a\) and Do\(^b\), and the high-prevalence antigens, Gy\(^a\), Hy, Jo\(^a\), DOYA, DOMR, and DOLG.\(^1,2\) The **DO** gene is located on chromosome 12p12.3, contains 3 exons, and encodes a protein comprised of 314 amino acids.\(^1,3\) The Do glycoprotein is an ADP-ribosyltransferase (CD297) that is linked via glycosylphosphatidylinositol (GPI) to the red blood cell (RBC) membrane.\(^1\) The glycoprotein is absent in the Do\(^null\) phenotype, known as Gy\((a–)\), which is rarely found in persons of white, black, Japanese, and Chinese populations.\(^2\) The Gy\((a–)\) phenotype is not associated with pathology, although the RBCs of patients with paroxysmal nocturnal hemoglobinuria Type III lack the Do glycoprotein.\(^3\) Dombrock antibodies are typically IgG-restricted, weakly reactive, and do not activate complement.\(^2\) Anti-Do\(^a\) and -Do\(^b\) can cause acute and delayed hemolytic transfusion reactions, but not hemolytic disease of the fetus and newborn (HDFN).\(^2\) Rare literature reports about anti-Jo\(^a\) exist, linking this specificity to delayed hemolytic transfusion reactions (DHTTRs).\(^1,5\)

The Jo\((a–)\) phenotype is caused by the 350C>T nucleotide substitution, causing an amino acid change from Thr to Ile at amino acid position 117.\(^1\) To date, only rare African-American individuals have been identified with the Jo\((a–)\) phenotype, although more than 99 percent of these individuals express Jo\(^b\).\(^2\) RBC genotyping of ethnic groups in West, Central, and East Africa revealed the allele frequencies for **DO*01.–05**, the Jo\((a–)\) phenotype, to be as high as 15 percent.\(^6\) Jo\(^a\) and Hy (ISBT [International Society of Blood Transfusion] allele **DO*02.–04**) show a phenotypical relationship. RBCs that are Jo\((a–)\) have weak reactivity with anti-Do\(^a\), no or weak reactivity with anti-Do\(^b\), reactivity with anti-Gy\(^a\), and weak reactivity with anti-Hy.\(^2\) RBCs that are Hy– often type as Jo\((a–)\), and the proximity of Hy (amino acid 108) and Jo\(^a\) (amino acid 117) likely explains this phenomenon.\(^3\) Nevertheless, some Hy– RBCs have been shown to express weak Jo\(^a\).\(^7\) Patients who are Jo\((a–)\) may be either **DO*01.–05/DO*01.–05** or **DO*02.–04/DO*01.–05.**\(^2\) Because of past confusion of anti-Hy as anti-Jo\(^a\), it is best to use reagent RBCs tested by DNA analysis to confirm anti-Jo\(^a\) specificity.\(^2\)

**Case Report**

A 32-year-old African-American woman with a history of sickle cell disease (SCD) was seen for left total hip arthroplasty due to avascular necrosis of the femoral head. Blood type and an antibody detection test were ordered on the day of surgery. The patient’s RBCs typed as group O, D+. Previous records showed a known anti-Fy\(^a\), acquired from past transfusion. The
antibody identification panel showed weak to 1+ panreactivity with Fy(a–) cells. The patient was last transfused with RBCs 2 years earlier, was not on a chronic transfusion regimen, and had no history of transfusion reactions. The autocontrol and direct antiglobulin test (DAT) were negative, so a novel alloantibody was suspected. Because the antibody identification was inconclusive, a pre-transfusion blood sample was sent to a reference laboratory for antibody identification and RBC genotyping. Later that day, the surgery was performed with an estimated blood loss of 450 mL, reducing the hemoglobin (Hb) from 10.2 to 8.6 g/dL (reference range 14.0–18.0 g/dL). One unit of RBCs was ordered emergently. One unit of Fy(a–), C–, E–, K–, HbS– RBCs was selected, but was weakly crossmatch incompatible. The unit was released with a risk form with orders to “transfuse with caution.” The transfusion proceeded without incident, and the patient was discharged in stable condition.

Several days after discharge, the reference lab reported their findings. Anti-Fy(a–) and new anti-Jo– and anti-Jk(b–) were identified. The RBC unit transfused during the hospitalization was known to be Jk(b–). Thus, the crossmatch incompatibility was attributed to anti-Jo–, which targets a high-prevalence antigen found in 100 percent of most populations. The anti-Jo– was reactive by indirect antiglobulin test (IAT), polyethylene glycol (PEG)-IAT, and ficin-IAT. Genotyping predicted the patient’s RBCs to be Fy(a–b–), Do(a+b+), Jo(a–), and Hy+. In addition, the patient was homozygous for the Duffy null promoter FY*02N.01 and for RHCE*01.01, which is associated with altered expression of e and the presence of a variant e allele.8

The patient was non-compliant with her prophylactic medications, and 2 weeks after discharge, the patient was admitted to the hospital because of a sickle-related vaso-occlusive pain crisis. Although the patient was clinically stable, her Hb was 6.7 g/dL, so 1 unit of RBCs was emergently ordered. One unit of Fy(a–), Jk(b–), C–, E–, K–, HbS– RBCs, which was weakly crossmatch incompatible, was transfused. The crossmatch incompatibility was attributed to anti-Jo–. Several hours after completion of the transfusion, the patient’s temperature peaked at 38.4°C, which was 37.1°C pre-transfusion. On the following day, the Hb was unchanged. Lactic acid dehydrogenase (LDH) increased from 951 to 2464 U/L (reference range 120–246 U/L), potassium (K+) increased from 3.7 to 4.6 mEq/L (reference range 3.5–5.5 mEq/L), and creatinine (Cr) increased from 0.60 to 0.98 mg/dL (reference range 0.6–1.60 mg/dL). The clinical team suspected a hemolytic transfusion reaction. The patient was informed about the suspected transfusion reaction; however, the patient was upset and left the hospital against medical advice.

Discussion

This case report serves to contribute to the rare literature implicating anti-Jo– in DHTRs.1,5 Jo– is a high-prevalence antigen in the Dombrock blood group system, which is found in 100 percent of most populations. Only African-American individuals have been identified as being Jo(a–); although the antigen is still present in greater than 99 percent of individuals in this population. Because finding Jo(a–) units can be nearly impossible in an emergent setting, it is important to understand the clinical significance of anti-Jo–.

The patient presented in this case report demonstrated findings consistent with that known about the Jo(a–) phenotype. The patient was African American, and her RBCs typed as Do(a+b+) and Hy+. On two separate occasions, the patient was transfused with 1 unit of RBCs, each of which was weakly crossmatch incompatible, attributed to anti-Jo–. Transfusion of the first RBC unit was not immediately associated with symptoms of a transfusion reaction. However, 2 weeks later, the patient returned with an Hb of 6.7 g/dL. The patient received a second RBC unit that was weakly crossmatch incompatible, also attributable to anti-Jo–. On the following day, the Hb remained unchanged, LDH spiked, K+ and Cr increased, and the patient experienced a new-onset fever. These findings are consistent with a DHTR, occurring 2 weeks after transfusion of the first RBC unit. DHTRs typically occur days to weeks after transfusion and are mediated by non-brisk extravascular hemolysis. Patients may be asymptomatic, but with unexplained anemia. Fortunately, most DHTRs have a benign course and require only supportive care, with monitoring of the hematocrit.

In retrospect, the transfusion reaction was potentially avoidable. Both RBC transfusions could be categorized as overtransfusion because the patient was clinically stable before being transfused. On the day after the first transfusion of 1 RBC unit, the patient was discharged because she was clinically stable, just as she had been prior to transfusion. In addition, the second transfusion of 1 RBC unit could have been avoided. In general, patients with SCD who are clinically stable with a high reticulocyte count do not need RBC transfusions.9 This patient was in sickle pain crisis in stable condition with a 6.7 g/dL Hb and reticulocyte count of 11.9%. From the perspective of judicious transfusion medicine practice, the risk of a hemolytic transfusion reaction from a crossmatch-incompatible RBC unit outweighed the potential benefits.
Other transfusion management options could have been considered in a non-urgent/non-emergent setting. Rare donor registries, such as the American Rare Donor Program (ARDP), are an effective tool for finding antigen-negative RBC units. Although they are even more rare than Jo(a–) units, Gy(a–) units would also be compatible because Gy(a–) represents the Do\null phenotype. Family members of the patient could have been tested to identify those who were ABO compatible, Fy(a–), Jk(b–), C–, E–, K–, HbS–, and possibly Jo(a–). Another option to ensure the safety of transfusion therapy is the monocyte monolayer assay (MMA). This could have been used to predict the clinical significance of the anti-Jo\a. An MMA with a monocyte index of more than 5 percent would have supported avoidance of transfusion in this case. Lastly, hemoglobin-based oxygen carriers (HBOCs) may become an alternative therapy for patients requiring RBCs that are difficult to obtain. Some patients may be able to receive HBOCs through clinical trials.11

Conclusions

This case report presents findings that are consistent with a DHTR due to anti-Jo\a, a rare antibody of relatively unknown clinical significance. The transfusion medicine service should be aware that anti-Jo\a has been implicated in DHTRs. The risks and benefits of transfusing an incompatible unit should be assessed for each patient. Because Jo\a is a high-prevalence antigen, securing Jo(a–) blood can be nearly impossible in an urgent/emergent setting. In a non-emergent setting, accessing the ARDP, testing likely Jo(a–) blood donors, such as persons of African-American ethnicity and family members, and the use of hemoglobin-based oxygen carriers are possible options to avoid hemolytic reactions. Fortunately, several weeks after the transfusion reaction, the patient returned to our hospital as an outpatient in stable condition.

Acknowledgments

We would like to thank the blood bank staff at Augusta University Medical Center for their help with this case report. In addition, we would like to thank Cindy Piefer from BloodCenter of Wisconsin for helping us to interpret the complex laboratory findings.

References

Recognizing and resolving ABO discrepancies

G.M. Meny

Patient samples are routinely typed for ABO prior to transfusion. Determining the ABO group requires both red blood cell (RBC) antigen typing for A and B (forward type) and testing for anti-A and anti-B in the plasma (reverse type). An ABO discrepancy exists when the result of an ABO RBC typing, or forward type, does not agree with the result of the plasma typing, or reverse type. This brief review examines several causes of ABO discrepancies encountered in the clinical transfusion service. Options for resolving these discrepancies are presented, including a discussion of which discrepancies should be resolved using molecular testing. Finally, case studies illustrate transfusion options in patients with ABO discrepancies. Discrepancies can also be encountered when ABO typing is performed on samples from blood or tissue donors, although those discrepancies will not be discussed in this review. Immunohematology 2017;33:76–81.

Key Words: ABO blood group, ABO discrepancies, serologic typing, transfusion service

The ABO blood group system was first described in 1900 by Landsteiner when agglutination was noted after mixing red blood cells (RBCs) and sera together from several individuals.1 Landsteiner initially named the groups “A,” “B,” and “C” based on the RBC agglutination patterns. Terminology was later changed and group “C” became group “O,” which is more familiar to clinical laboratory scientists. Group AB, the fourth and final blood group of the system, was reported by von Decastello and Sturli in 1902.2

Their findings summarize the most important feature of the ABO blood group system: an ABO antibody (anti-A and/or anti-B) is almost always present in an individual’s plasma when the corresponding antigen(s) is absent from their RBCs.2 This “double check” in determining an individual’s ABO group provides the ability to obtain a forward ABO type (patient RBCs typed using commercial antisera) and compare it with the reverse ABO type (patient plasma typed using commercial RBCs) (Table 1). The forward and reverse typing results should agree. An ABO discrepancy exists if they do not. Clinically, one should consider the potential for a worst-case scenario adverse event when transfusion is requested for a patient with an ABO discrepancy.

The purpose of this brief review is to examine several causes of ABO discrepancies encountered in the clinical transfusion service. Options for resolving these discrepancies will be presented, including a discussion of which discrepancies should be resolved using molecular testing. Finally, case studies are provided to illustrate transfusion options in patients with ABO discrepancies. Discrepancies can also be encountered when ABO typing is performed on samples from blood or tissue donors, but those discrepancies will not be discussed.

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RBCs = red blood cells.

ABO Discrepancy Resolution: General Approach

An ABO discrepancy exists when the result of an ABO RBC typing, or forward type, does not agree with the result of the plasma typing, or reverse type. It is extremely important in the clinical laboratory to record these initial discrepant results. The following list summarizes a general approach that can be followed when an ABO discrepancy is encountered:

1. Repeat the test with the same sample.
2. Repeat the test with a new sample.
3. Review the patient’s medical record.
4. Review prior in-house laboratory testing records.
5. Contact other healthcare facilities.

Repeat the test from the beginning using the same sample because plasma and/or RBC suspensions can be inadvertently mixed up within the laboratory.3 Results and interpretations may also be mistakenly recorded. When repeating the testing, prepare a fresh RBC suspension, since a mix-up in sample identification may have occurred during initial testing. Most laboratories perform initial forward typing using normal saline–washed RBCs. Use normal saline–washed RBCs if initial typing was performed using plasma-suspended RBCs. If the repeat results are the same as the first, request a new sample for testing.
ABO Discrepancies: Causes and Resolutions

Technical Problems

Technical problems should be ruled out with a check of reagents and equipment and repeating the testing. Examples of technical problems include a failure to follow standard operating procedure or commercial package inserts, improper centrifugation, improper preparation of RBC suspensions, or lack of addition of reagents. Appropriate medical and supervisory staff should be notified if technical problems are suspected, especially sample misidentification (see Discussion).

If technical problems are ruled out, one should then consider an intrinsic problem with either the RBCs and/or plasma as the cause of the discrepancy. For the sake of convenience and ease of resolution, discrepancies can be categorized as to whether the unexpected results occur in the forward or reverse typing (Fig. 1). The following sections provide further details regarding investigating and resolving forward (RBC) and reverse (plasma) discrepancies.

Red Blood Cell Discrepancy: Weak or Missing Reactivity

Forward ABO typing is performed using commercial antisera combined with patient RBCs, and this testing typically results in strong RBC agglutination. One should consider a potential ABO discrepancy when weaker than expected RBC agglutination is obtained (Table 2). Possible causes for weak or missing RBC reactivity include the following: A or B subgroups, recent transfusion, stem cell transplant, and leukemia or malignancy.

Table 2. Examples of ABO discrepancies

<table>
<thead>
<tr>
<th>Patient RBCs and Plasma and</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A, RBCs</th>
<th>B RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC weak/missing reactivity</td>
<td>2+</td>
<td>0</td>
<td>0–2+</td>
<td>4+</td>
</tr>
<tr>
<td>RBC extra reactivity</td>
<td>2+</td>
<td>3+</td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>RBC mixed-field reactivity</td>
<td>3+; mf</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>Plasma weak/missing reactivity</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>Plasma extra reactivity</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>1+</td>
</tr>
</tbody>
</table>

RBCs = red blood cells; mf = mixed field.

Note that in the RBC weak/missing reactivity example provided in Table 2, less than 3+ agglutination is obtained when patient RBCs are typed with anti-A. This finding may be seen in individuals with A subgroups. More frequently, individuals with A subgroups (or A subgroup B) are detected because of a plasma discrepancy when anti-A1 is detected (see Plasma Discrepancy: Extra Plasma Reactivity). The variation in agglutination reactivity occurs because of differences in amounts of antigen present on RBCs or in secretions. Subgroups of A are more common than subgroups of B. The two most important subgroups clinically are A1 and A2. A1 is distinguished by its reactivity with the lectin, Dolichos biflorus (anti-A1–like reactivity). For the sake of simplicity in this review, all other A subgroups nonreactive with Dolichos biflorus are referred to as “A1.” In addition, subgroup A2 shows increased reactivity with lectin, Ulex europaeus (anti-H–like reactivity). Subgroup A1 is nonreactive with Ulex europaeus.

Other methods are available that may prove of assistance in resolving discrepancies due to weak or missing RBC reactivity. Refer to the references and manufacturer’s directions for additional information. For example, forward ABO typing can be repeated using other monoclonal antisera, by increasing the incubation time, or by using enzyme-treated RBCs. Saliva studies can be performed to look for the presence of ABH antigens using inhibition methods because ABH antigens that are undetectable on the RBC surface may be detectable in the saliva of secretors. Molecular testing is also
available to identify an individual’s ABO genotype. However, molecular testing is seldom necessary for clinical use in resolving most common ABO discrepancies, and care must be taken to understand the potential for genotype/phenotype discrepancies\(^5,10\) (see Discussion).

**Red Blood Cell Discrepancy: Extra RBC Reactivity**

This discrepancy appears when unexpected or “extra” reactivity is detected in the forward typing. In the example provided in Table 2, the patient’s blood group likely would be interpreted as group B except that there is 2+ unexpected or “extra” reactivity with the patient’s RBCs and anti-A in the forward type. Possible causes of an ABO discrepancy due to extra RBC reactivity include a recent out-of-group transfusion, stem cell transplantation, rouleaux, an antibody to a reagent constituent, acquired B, and cold or warm autoantibodies.

When an ABO discrepancy due to extra RBC reactivity is suspected, check the patient’s medical record or speak with the patient or his or her clinician to determine if a recent transfusion or stem cell transplant has occurred. Recent transfusion with non–ABO-identical RBCs should result in a mixed-field typing result (see Red Blood Cell Discrepancy: Mixed-Field Reactivity), although interpretation may be difficult. Similar results may be noted post-transplant with non–ABO-identical stem cells. Rouleaux or an antibody to the antigen as a cause of extra RBC reactivity will likely only be seen if the patient’s RBCs are suspended in plasma prior to performing the forward type. In this situation, the discrepancy can be resolved by washing and re-suspending patient RBCs in saline, followed by repeat forward typing with anti-A and anti-B. Acquired B is a rare event. Patients not only must inherit or acquire this condition, but antisera must detect these RBC membrane modifications. Methods are available to remove cold and warm autoantibodies from RBCs.\(^5,12\) Follow the manufacturer’s directions if a commercial kit is used.

**Red Blood Cell Discrepancy: Mixed-Field Reactivity**

A RBC typing result that appears to contain RBCs from more than one ABO group is known as a “mixed-field reaction.” Using a gel method, unagglutinated RBCs appear at the bottom of the column, while agglutinated RBCs are detected at the top of the column. In Table 2, mixed-field reactivity was noted when the patient’s RBCs were typed with anti-A, and the results could be interpreted as either group A or group O. The reverse typing is consistent with group A. Possible causes of an ABO discrepancy due to mixed-field RBC reactivity include recent out-of-group transfusion, stem cell transplantation, A subgroup (especially A\(_3\) subgroup), fetomaternal hemorrhage, and chimerism.

Check the patient’s medical record or speak with the patient or the patient’s clinician to determine if a recent out-of-group transfusion or stem cell transplant has occurred. If a large fetomaternal hemorrhage is the suspected cause of the mixed-field reactivity, a medical record review of the mother and newborn can also be of assistance. If mixed-field reactivity is uncertain, examine the RBC sample for the presence of mixed-field reactivity in other blood group antigens when typed using serologic methods. Cell separation methods may be useful to permit patient phenotyping when recent transfusion has occurred (reticulocyte separation method\(^12\)) or via hypotonic lysis for patients with sickle cell disease who have recently been transfused.\(^13\)

**Plasma Discrepancy: Weak or Missing Reactivity**

Reverse ABO typing uses commercial RBCs combined with patient plasma. The agglutination observed from patient anti-A and anti-B may not consistently be as strong as that seen when using commercial antisera to type the patient RBCs in the forward ABO procedure. Thus, a reverse ABO typing method, such as tube, microplate, or column agglutination, should be selected that will adequately detect plasma anti-A and anti-B.\(^5\) Weak agglutination results should cause one to consider a potential ABO discrepancy prior to interpreting an ABO group (Table 2). Possible causes of weak or missing plasma reactivity include immunosuppression, post–stem cell transplantation, hypogammaglobulinemia, and age-related changes in ABO isoagglutinin levels.

A medical record review may assist in the initial evaluation of this discrepancy. Note the age of the patient. ABO isoagglutinins are usually initially detected around age 3 months and reach adult levels between age 5 and 10 years. Antibody titers vary considerably between individuals and decline with age.\(^14\) Note the patient’s diagnosis and treatment plan. Consider the possibility of hypogammaglobulinemia caused either by an inherited immune disorder or secondary to another medical condition or medication (such as rituximab). Cold temperature incubation may be used to enhance detection of weak ABO antibodies, provided appropriate controls to detect cold autoantibodies and alloantibodies are included.\(^6\)

**Plasma Discrepancy: Extra Plasma Reactivity**

Analogous to discrepancies secondary to extra RBC reactivity, these plasma discrepancies appear when unexpected or “extra” reactivity is detected in the reverse typing. In the example provided (Table 2), the patient’s blood
group likely would be interpreted as group B except that there is 1+ unexpected or extra reactivity with the patient’s plasma and the reagent B cells in the reverse type. Possible causes of an ABO discrepancy due to extra plasma reactivity include ABO subgroup, cold reactive alloantibody, cold reactive autoantibody, antibody to reagent constituent, transfusion of non-ABO-group-specific plasma components, infusion of IVIG, and post–stem cell transplant status.

A medical record review, speaking with the patient’s clinical team, or speaking with the patient may assist in the initial evaluation of this discrepancy. Has the patient received a component or derivative that could provide information to explain the extra plasma reactivity? For example, were non–group-specific plasma-containing components transfused? Were derivatives infused? Has the patient ever been diagnosed with leukemia or any type of malignancy? Has the patient received a transplant, and if so, what type (stem cell or solid organ), and what was the donor’s blood group?

Because forward RBC typing is performed using commercial typing antisera, ABO subgroups are frequently recognized because of a discrepancy between RBC forward and plasma reverse typing results. Anti-A1 in A2 individuals is a common discrepancy that can occur in 1–8 percent of group A2 and 22–35 percent of group A2B individuals. The strength of reactivity between the anti-A1 with the A1 reagent RBCs is usually weaker than that noted between anti-B and B reagent RBCs. Additional room temperature tests can be performed to assist in resolving this discrepancy. In general, the following testing is generally sufficient to identify most group A subgroups with anti-A1:

- RBCs tested with anti-A1 lectin = negative
- Plasma tested with A1 and O RBCs = negative
- Plasma tested with A1 RBCs = positive

Unexpected anti-B can also occur in B subgroups, such as Bx. These subgroups are not as common as A subgroups. Note that it is not important to identify or categorize the subgroup for clinical management.

**Summary**

The information from this section and test results should provide clues as to the source of the extraneous plasma reactivity. Causes other than ABO antibodies should be identified if testing with the A1, O, and/or autologous RBCs is positive. A positive antibody detection test at room temperature may be caused by a cold autoantibody (anti-I, anti-IH, etc.), cold alloantibody (anti-M, -N, -Lea, -P1, etc.), or an antibody to a reagent constituent. An antibody to a reagent constituent can be concluded if negative results are obtained using washed panel and reverse grouping cells. If a RBC alloantibody is identified, repeat the reverse ABO testing using antigen-negative reverse RBCs. If a RBC autoantibody is identified, repeat the reverse typing using cold auto-adsorbed plasma, if possible. Rabbit erythrocyte stroma–adsorbed plasma should not be used to determine reverse ABO group as anti-B, in addition to other RBC alloantibodies, can be removed during the adsorption process.

**Case Studies**

**Patient 1 and Patient 2**

Two patients (patient 1 and patient 2) presented on the same day for preoperative evaluation. Both patients’ ABO testing results were identical (Table 3). The forward typing results were consistent with group A, but the reverse typing results appeared to demonstrate extra plasma reactivity with the A1 reagent RBCs. Plasma reactivity with the A1 RBCs was suspected to be extraneous, since it is weaker than would be expected in a group O individual; thus, the ABO group was not believed to be group A, and a discrepancy was investigated noting extra plasma reactivity between reagent A1 RBCs and patient plasma.

**Table 3. Initial test results, patients 1 and 2**

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>Anti-D</th>
<th>Rh Control</th>
<th>A, RBCs</th>
<th>B, RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>4+</td>
<td>0</td>
<td>NT</td>
<td>3+</td>
<td>0</td>
<td>1+</td>
<td>4+</td>
</tr>
<tr>
<td>Patient 2</td>
<td>4+</td>
<td>0</td>
<td>NT</td>
<td>3+</td>
<td>0</td>
<td>1+</td>
<td>4+</td>
</tr>
</tbody>
</table>

RBCs = red blood cells; NT = not tested.

The tests were repeated to rule out technical issues, and identical results were obtained. A review of the medical records for both patients provided no significant information. Both patients were male and scheduled for elective joint replacement surgery. Current medications included atorvastatin, hydrochlorothiazide, enalapril, and metoprolol. Neither patient had a history of prior transfusions, infusion of IVIG, or stem cell transplant.

Because extraneous plasma reactivity was suspected, both patients’ RBCs were initially tested with Dolichos biflorus, and their plasma was tested at room temperature against group O reagent screening RBCs, autologous RBCs, group A, and group A2 RBCs for the presence of anti-A1, and for cold-reactive alloantibodies and autoantibodies.

The RBCs of patient 1 demonstrated no agglutination with Dolichos biflorus. The plasma of patient 1 agglutinated two examples of A1 RBCs but did not agglutinate two examples of A2 RBCs. No agglutination was observed with the group...
O screening RBCs. Thus, the final ABO interpretation for patient 1 is consistent with an “A subgroup (probable A1) with anti-A1.” Note that there were no room temperature–reactive RBC alloantibodies present; thus, random group A, A2, and B RBCs were used to perform the reverse typing.

Anti-A1 is considered clinically significant if reactivity is observed at 37°C. If reactivity is observed at 37°C, group O or A2 RBCs can be transfused to A1 individuals, and group O, A2, or A1B RBCs can be transfused to A1B individuals.5 Some transfusion services issue only group O RBCs to patients with anti-A1 to avoid an incompatible crossmatch or to avoid screening units.

The RBCs of patient 2 also demonstrated no agglutination with Dolichos biflorus, although the plasma of patient 2 weakly agglutinated two examples of A1 RBCs and one example of A2 RBCs. In addition, screening cell 1 was positive at immediate spin and negative at the indirect antiglobulin test. The antibody detection test was interpreted as “positive”, and an antibody panel detected an anti-M reactive at immediate spin and room temperature. ABO reverse testing was repeated with two examples each of M– A1, A2, and B RBCs, revealing negative results with both group A1 and A2 RBCs and 4+ reactivity with group B RBCs. Thus, the final ABO interpretation is consistent with “group A (with anti-M).” Note that although this individual is also an A subgroup, anti-A1 is not present.

For patients with anti-M, crossmatch-compatible RBCs can be transfused when reactivity is observed at 37°C.18 Some transfusion services issue M– RBCs in scenarios such as that of patient 2 to avoid an incompatible immediate spin crossmatch.

**Patient 3**

Patient 3 was a 26-year-old woman who presented to the emergency department with chills and fever (38°C) and a petechial rash noted on her extremities. A complete blood count and type and screen were ordered. Initial type and screen results noted mixed-field positive agglutination with patient RBCs and reagent anti-B on forward type and 1+ agglutination with patient plasma and reagent B RBCs on reverse type. The same results were obtained upon repeat testing (Table 4).

This patient could have been considered a B subgroup. Additional history, however, revealed that the patient was post–stem cell transplantation (patient’s native ABO group was group A and donor was group B), and the patient received multiple RBC and platelet transfusions. The historical information was also consistent with the patient’s presentation of a potential septic episode, which is a known complication in a stem cell transplant patient.

Blood component selection for patients post–stem cell transplant is standardized to provide optimum conditions for erythropoietic progenitor engraftment.19 This patient most likely received group O RBCs and group AB platelets or plasma, which also could have contributed to the unusual ABO testing results.

**Discussion**

Serologic methods and information available within many hospital-based transfusion service laboratories can be used in resolving most ABO discrepancies. Discrepancies can be categorized into those involving the forward (RBC) type or the reverse (plasma) type. This arbitrary categorization helps to focus the search for a cause of the discrepancy. Yudin and Heddle20 devised a question-based approach to resolving discrepancies in a transfusion service laboratory. Although their publication20 focuses on serologic issues involved in discrepancies, several of the 13 questions listed in their investigative approach are useful and should be part of any ABO discrepancy investigation (e.g., What is the patient’s diagnosis? What is the transfusion history?).

Unfortunately, ABO discrepancies persist secondary to technical issues, especially sample misidentification (wrong blood in tube [WBIT]).21,22 Suspected WBIT requires prompt investigation per institution protocol because other laboratory samples may be involved. Appropriate supervisory staff should be notified as soon as technical difficulties are suspected. Medical director notification is also imperative because plans can be developed regarding transfusion needs, if necessary, especially if transfusion is emergent. Medical directors can also be of assistance in working with the laboratory and clinical staff and patients to provide updates if blood component delays or additional sample requests will be forthcoming.

Molecular methods are another tool that may be of use in resolving ABO discrepancies. Molecular methods are routinely being implemented for use in resolving, for example, Rh system discrepancies,23 yet clinical information is not yet available to make recommendations regarding widespread use of ABO genotyping for initial discrepancy resolution.24 Infrequent causes of discrepancies where molecular methods can be useful in resolving an ABO discrepancy include suspected chimerism. These individuals frequently demonstrate mixed-
field RBC reactivity. Chimerism can occur either from the fusion of more than one zygote (dispermic chimerism), when hematopoietic stem cells are shared, such as between twins in utero when placental blood vessels form anastomoses (twin chimerism), or post–stem cell transplant. If molecular testing is performed for ABO discrepancy resolution, care must be taken to understand the limitations of the test system used as well as factors leading to potential genotype/phenotype discrepancies.

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1. Reid ME, Shine I. In the beginning was the ABC. In: The discovery and significance of the blood groups. Cambridge, MA: SBB Books, 2012:3–10.

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Bloody Brilliant: A History of Blood Groups and Blood Groupers

S. Gerald Sandler

What a joy and privilege to read and reread this unique and extraordinarily informative history for this review! Pierce and Reid have authored a 633-page, 28-chapter tome, containing 796 illustrations, including photographs of individual contributors to the field of blood group serology, as well as group photographs of landmark meetings and conferences held during the past 100 years. The Index lists the names of 1046 individuals who are acknowledged as contributors to the history of blood group serology, many of whom are the subject of cameo biographies.

First and foremost, Bloody Brilliant is a meticulously documented history of the discovery and early characterization of blood groups, beginning with Karl Landsteiner and the ABO blood group system at the beginning of the 20th century. Each chapter concludes with an extensive list of references. There are a total of 5014 journal articles and other citations, making this volume a must-go-to resource for any future blood group system review that intends to be comprehensive and include the early discoveries and studies. Bloody Brilliant is accompanied by formal portraits of individual contributors, including a studio-quality full-page portrait of Karl Landsteiner and another of Philip Levine with Alexander Wiener. For the early history, Pierce and Reid conducted a global search for suitable illustrations and portraits. Each photograph is credited to its source, including the Wellcome Library (London), the University Archives (Heidelberg), the National Portrait Gallery (London), the Archivo Storico dell’Università deli Studi (Pavia), the University of Debrecen Electronic Archive (Debrecen), the Max Planck Society (Berlin), the State Library of South Australia (Adelaide), and many others. The authors recognize the historic role of organizations such as the AABB, the American Red Cross, and the Invitational Conference of Investigative Immunohematologists (ICII, colloquially known as “Icky”), as well as the support of specific hospitals, blood centers, and reagent manufacturers that maintained reference laboratories and fostered generations of expert reference serologists.

In addition to a formal history of blood group serology, this volume is a scrapbook of snapshots, personal recollections, and intimate profiles of the personalities behind the journal articles and conference presentations. An alternative subtitle for Bloody Brilliant might have been “Blood Groupers After Hours.” From early years to the present, the discipline of blood group serology has consisted of a relatively small number of serious professionals who shared their scientific work openly and who collaborated at an international level, sharing ideas, blood samples, lecture slides, and bar tabs. Bloody Brilliant captures this unique international community with anecdotes and illustrations. There is a nine-page ICII Photo Album with 89 snapshots contributed by Kay Beattie, Don Swanson, Marilyn Moulds, Ellen Case (for John Case), Steve Pierce, and others. The ICII Photo Album captures informal portraits and scenes from four decades of meetings, after-meetings, and the casual side of blood groupers around the globe.
Lastly, this tome requires a production model for the publisher, AABB Press, that is unique among their publications. Typically, AABB Press publications—Standards, Technical Manual, Guidelines, and scientific texts—have a short life, becoming outdated only months to a few years after printing. Bloody Brilliant, like good wine, will only increase in value over time. It will never go out of date. As the authors state in the Preface, “newcomers to our field will not personally know the giants and the many anecdotes and behind-the-scenes stories that we had been raised on [and are] about to be lost.” A few years from now, the next generation of blood groupers—probably molecular scientists—will need to seek out copies of Bloody Brilliant to understand the foundation of the discipline. I am hopeful that AABB Press will keep Bloody Brilliant available for the long term. When our generation of blood groupers retires, discarding our outdated textbooks and conference proceedings, we’ll keep our copies of Bloody Brilliant for our personal libraries. Bloody Brilliant captures a uniquely personal, collegial, and productive history. It is a valued resource for our generation and will be for generations to follow.

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The Department of Transfusion on Medicine Specialist in Blood Bank Technology Program

The National Institutes of Health (NIH) Clinical Center, America’s research hospital, is located on the NIH campus in Bethesda, Maryland. Through clinical research, clinician-investigators translate laboratory discoveries into better treatments, therapies, and interventions to improve the nation’s health.

The Department of Transfusion Medicine (DTM) Specialist in Blood Bank (SBB) Training Program was established in 1966. Many of its graduates are now technical supervisors, education coordinators, quality assurance specialists, or reference technologists at some of the nation’s finest blood banks and transfusion services. Others have joined commercial companies in reference and education capacities. The program is a 1-year course (July–July) in advanced blood bank technology. The NIH Clinical Center Blood Bank, SBB Technology Program is accredited by the Commission on Accreditation of Allied Health Education Programs (www.caahep.org) upon the recommendation of the AABB Committee on Accreditation of SBB Programs.

The curriculum includes formal and informal teaching sessions covering basic and advanced serological techniques, blood donations, genetics, molecular immunohematology, viral disease testing, blood preservation and storage, component therapy, apheresis, hazards of transfusion, immunology, human leukocyte antigen (HLA) and transplantation, blood bank administration, and other relevant topics. Participation is encouraged at monthly departmental blood bank Journal Club presentations, Laboratory Services Section’s continuing education opportunities, and weekly transfusion medicine conferences. Students complete rotations in the DTM Laboratory Services Section, which includes an AABB-Accredited Immunohematology Reference Laboratory and an ASHI-accredited HLA Typing Laboratory. A rotation in the Blood Services Section of the DTM provides experience in donor recruitment, screening, phlebotomy, and apheresis procedures. Experience in infectious disease testing and hematopoietic transplantation is obtained through rotations in both the Infectious Diseases and Cell Processing Sections of the DTM. Rotations off-site address neonatal/pediatric transfusion practices and specialized testing procedures, such as IgA and monocyte monolayer assays.

With the guidance of a senior DTM staff member, each student is required to pursue an in-depth project suitable for presentation and/or publication. The project may concern a research issue in blood banking, a case study with a review of the literature, or an educational project.

Interested applicants should contact:

Karen M. Byrne, MDE, MT(ASCP)SBB
NIH Clinical Center, Department of Transfusion Medicine
Building 10, Room 1C711
10 Center Drive, MSC 1184
Bethesda, MD 20892-1184
Phone: 301-451-8645
Kbryne@cc.nih.gov
A must for the bookshelf of every blood bank. A superb and beautiful book.

John Gorman MD, Lasker Award 1980

The authors convey the excitement of scientific discovery so effectively on every page.

S. Gerald Sandler, MD
Professor of Medicine and Pathology,
Georgetown University Hospital

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Phyllis Walker, MT(ASCP)SBB,
San Francisco

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Sandra J. Nance

The most valued and useful resource in my blood banking and immunohematology library . . . it is highly readable and an enjoyable, painless way to update your information about blood group antigens.

Immunohematology

Blood Group Antigens & Antibodies
by Marion Reid & Christine Lomas-Francis
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or visit
www.bloodgroups.info
www.sbbpocketbook.com
**What is a certified Specialist in Blood Banking (SBB)?**
- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

**Individuals who have an SBB certification serve in many areas of transfusion medicine:**
- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in transfusion medicine
- Conduct research in transfusion medicine

**Who are SBBs?**
- Supervisors of Transfusion Services
- Managers of Blood Centers
- Supervisors of Reference Laboratories
- Research Scientists
- Quality Assurance Officers
- Technical Representatives
- LIS Coordinators
- Consumer Safety Officers
- Educators
- Reference Lab Specialists

**Why become an SBB?**
- Professional growth
- Job placement
- Job satisfaction
- Career advancement

**How does one become an SBB?**
- Attend a CAAHEP-accredited SBB Technology program **OR**
- Sit for the examination based on criteria established by ASCP for education and experience.

**However:** In recent years, a greater percentage of individuals who graduate from CAAHEP-accredited programs pass the SBB exam.

**Conclusion:** The **BEST** route for obtaining an SBB certification is . . . to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program.

Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org.

<table>
<thead>
<tr>
<th>State</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>American Red Cross Blood Services</td>
<td>Pomona, CA</td>
</tr>
<tr>
<td>Florida</td>
<td>Academic Center at OneBlood</td>
<td>St. Petersburg, FL</td>
</tr>
<tr>
<td>Illinois</td>
<td>Rush University</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Indiana</td>
<td>Indiana Blood Center</td>
<td>Indianapolis, IN</td>
</tr>
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<td>Louisiana</td>
<td>University Medical Center New Orleans</td>
<td>New Orleans, LA</td>
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<tr>
<td>Maryland</td>
<td>National Institutes of Health Clinical Center</td>
<td>Bethesda, MD</td>
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<td></td>
<td>The Johns Hopkins Hospital</td>
<td>Baltimore, MD</td>
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<tr>
<td></td>
<td>Walter Reed National Military Medical Center</td>
<td>Bethesda, MD</td>
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<tr>
<td>Texas</td>
<td>University Health System and Affiliates School of Blood Bank Technology</td>
<td>San Antonio, TX</td>
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<td></td>
<td>University of Texas Medical Branch</td>
<td>Galveston, TX</td>
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<tr>
<td>Wisconsin</td>
<td>BloodCenter of Wisconsin</td>
<td>Milwaukee, WI</td>
</tr>
</tbody>
</table>

Revised October 2016
Diagnostic testing for:
• Neonatal alloimmune thrombocytopenia (NAIT)
• Posttransfusion purpura (PTP)
• Refractoriness to platelet transfusion
• Heparin-induced thrombocytopenia (HIT)
• Alloimmune idiopathic thrombocytopenia purpura (AITP)

Medical consultation available

Test methods:
• GTI systems tests
  — detection of glycoprotein-specific platelet antibodies
  — detection of heparin-induced antibodies (PF4 ELISA)
• Platelet suspension immunofluorescence test (PSIFT)
• Solid-phase red cell adherence (SPRCA) assay
• Molecular analysis for HPA-1a/1b

For further information, contact:
Platelet Serology Laboratory (215) 451-4205
Sandra Nance (215) 451-4362
Sandra.Nance@redcross.org
American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594

National Reference Laboratory
for Specialized Testing

National Neutrophil Serology Reference Laboratory

Our laboratory specializes in granulocyte antibody detection and granulocyte antigen typing.

Indications for granulocyte serology testing include:
• Alloimmune neonatal neutropenia (ANN)
• Autoimmune neutropenia (AIN)
• Transfusion-related acute lung injury (TRALI)

Methodologies employed:
• Granulocyte agglutination (GA)
• Granulocyte immunofluorescence by flow cytometry (GIF)
• Monoclonal antibody immobilization of neutrophil antigens (MAINA)

TRALI investigations also include:
• HLA (PRA) Class I and Class II antibody detection

For further information, contact:
Neutrophil Serology Laboratory (651) 291-6797
Randy Schuller (651) 291-6758
Randy.Schuller@redcross.org
American Red Cross Biomedical Services
Neutrophil Serology Laboratory
100 South Robert Street
St. Paul, MN 55107

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Reference and Consultation Services

Antibody identification and problem resolution
- HLA-A, B, C, and DR typing
- HLA-disease association typing
- Paternity testing/DNA

For information, contact:
Mehdizadeh Kashi
at (503) 280-0210
or write to:
Tissue Typing Laboratory
American Red Cross Biomedical Services
Pacific Northwest Region
3131 North Vancouver
Portland, OR 97227

CLIA licensed, ASHI accredited

IgA Testing

IgA testing is available to do the following:
- Identify IgA-deficient patients
- Investigate anaphylactic reactions
- Confirm IgA-deficient donors

Our ELISA for IgA detects protein to 0.05 mg/dL.

For additional information contact:
Sandra Nance (215) 451-4362
or e-mail:
Sandra.Nance@redcross.org
or write to:
American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Sandra Nance

IgA Testing
- Effective tool for screening large volumes of donors
- Gel diffusion test that has a 15-year proven track record:
  Approximately 90 percent of all donors identified as IgA deficient by this method are confirmed by the more sensitive testing methods

For additional information:
Kathy Kaherl
at (860) 678-2764
e-mail:
Katherine.Kaherl@redcross.org
or write to:
Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Avenue
Farmington, CT 06032

National Reference Laboratory for Blood Group Serology

Immunohematology Reference Laboratory
AABB, ARC, New York State, and CLIA licensed
24-hour phone number:
(215) 451-4901
Fax: (215) 451-2538

American Rare Donor Program
24-hour phone number:
(215) 451-4900
Fax: (215) 451-2538
ardp@redcross.org

Immunohematology
Phone, business hours:
(215) 451-4902
Fax: (215) 451-2538
immuno@redcross.org

Quality Control of Cryoprecipitated–AHF
Phone, business hours:
(215) 451-4903
Fax: (215) 451-2538
B. Preparation
1. Title: Allele Name (Allele Detail)
   - RHCE*01.01 (RHCE*ce48C)
2. Author Names (initials and last name of each [no degrees, ALL CAPS])

C. Text
1. Case Report
   i. Clinical and immunohematologic data
   ii. Race/ethnicity and country of origin of proband, if known
2. Materials and Methods
   Description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patient names or hospital numbers.
3. Results
   Complete the Table Below:

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Allele Name</th>
<th>Nucleotide(s)</th>
<th>Exon(s)</th>
<th>Amino Acid(s)</th>
<th>Allele Detail</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>e weak</td>
<td>RHCE*01.01</td>
<td>48G&gt;C</td>
<td>1</td>
<td>Trp16Cys</td>
<td>RHCE*ce48C</td>
<td>1</td>
</tr>
</tbody>
</table>

   Column 1: Describe the immunohematologic phenotype (ex. weak or negative for an antigen).
   Column 2: List the allele name or provisional allele name.
   Column 3: List the nucleotide number and the change, using the reference sequence (see ISBT Blood Group Allele Terminology Pages for reference sequence ID).
   Column 4: List the exons where changes in nucleotide sequence were detected.
   Column 5: List the amino acids that are predicted to be changed, using the three-letter amino acid code.
   Column 6: List the non-consensus nucleotides after the gene name and asterisk.
   Column 7: If this allele was described in a meeting abstract, please assign a reference number and list in the References section.

4. Additional Information
   i. Indicate whether the variant is listed in the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/); if so, provide rs number and any population frequency information, if available.
   ii. Indicate whether the authors performed any population screening and, if so, what the allele and genotype frequencies were.
   iii. Indicate whether the authors developed a genotyping assay to screen for this variant and, if so, describe in detail here.
   iv. Indicate whether this variant was found associated with other variants already reported (ex. RHCE*ce48C,1025T is often linked to RHD*DIVa-2).

D. Acknowledgments

E. References

F. Author Information
   List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable.
I. GENERAL INSTRUCTIONS
Before submitting a manuscript, consult current issues of Immunohematology for style. Number the pages consecutively, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW
A. Each component of the manuscript must start on a new page in the following order:
1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

B. Preparation of manuscript
1. Title page
   a. Full title of manuscript with only first letter of first word capitalized (bold title)
   b. Initials and last name of each author (no degrees; ALL CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
   c. Running title of ≤40 characters, including spaces
   d. Three to ten key words
2. Abstract
   a. One paragraph, no longer than 300 words
   b. Purpose, methods, findings, and conclusion of study
3. Key words
   a. List under abstract
4. Text (serial pages): Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections
   a. Introduction — Purpose and rationale for study, including pertinent background references
   b. Case Report (if indicated by study) — Clinical and/or hematologic data and background serology/molecular
   c. Materials and Methods — Selection and number of subjects, samples, items, etc., studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patients’ names or hospital numbers.
   d. Results — Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
   e. Discussion — Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
5. Acknowledgments: Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
6. References
   a. In text, use superscript, Arabic numbers.
   b. Number references consecutively in the order they occur in the text.
7. Tables
   a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of…) and use no punctuation at the end of the title.
   b. Use short headings for each column needed and capitalize first letter of first word. Ommit vertical lines.
   c. Place explanation in footnotes (sequence: *, †, ‡, §, ¶, **, ††).
8. Figures
   a. Figures can be submitted either by e-mail or as photographs (5 ×7” glossy).
   b. Place caption for a figure on a separate page (e.g., Fig. 1 Results of…), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.
   c. When plotting points on a figure, use the following symbols if possible:

III. EDUCATIONAL FORUM
A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:
1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem-solving skills
2. Annotated conference proceedings

B. Preparation of manuscript
1. Title page
   a. Capitalize first word of title.
   b. Initials and last name of each author (no degrees; ALL CAPS)
2. Text
   a. Case should be written as progressive disclosure and may include the following headings, as appropriate:
      i. Clinical Case Presentation: Clinical information and differential diagnosis
      ii. Immunohematologic Evaluation and Results: Serology and molecular testing
      iii. Interpretation: Include interpretation of laboratory results, correlating with clinical findings
      iv. Recommended Therapy: Include both transfusion and nontransfusion-based therapies
      v. Discussion: Brief review of literature with unique features of this case
      vi. Reference: Limited to those directly pertinent
      vii. Author information (see II.B.9.)
      viii. Tables (see II.B.7.)

IV. LETTER TO THE EDITOR
A. Preparation
1. Heading (To the Editor)
2. Title (first word capitalized)
3. Text (written in letter [paragraph] format)
4. Author(s) (type flush right; for first author: name, degree, institution, address [including city, state, ZIP code, and country]; for other authors: name, degree, institution, city and state)
5. References (limited to ten)
6. Table or figure (limited to one)

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Degree(s) ___________________________

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