Case report: exacerbation of hemolytic anemia requiring multiple incompatible RBC transfusions

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RBC transfusions in a patient with a history of autoimmune hemolytic anemia (AIHA) can represent both a laboratory and a clinical challenge. The development of high-titer low-avidity antibodies and antibodies to high-frequency antigens may further impair the ability to identify compatible donor RBCs. Not infrequently, incompatible RBCs must be used and the desire to increase oxygen carrying capacity conflicts with the desire to avoid exacerbating the autoimmune hemolytic process with RBC transfusions. A 66-year-old Caucasian female with coronary artery disease and a history of refractory AIHA had recently developed anemia and required multiple RBC transfusions. The patient had maintained adequate RBC counts with erythropoietin and prednisone therapy for the previous 16 months. With the recent worsening of her hemolytic anemia, she had developed angina that was treated with RBC transfusions in an outpatient setting. However, her angina increased as her RBC counts decreased, leading to hospital admission for further management of her hemolytic anemia and angina. She subsequently required multiple incompatible RBC transfusions despite increased prednisone therapy and did not improve until after coronary artery stent placement and high dose IVIG therapy. This case demonstrates the usefulness of early patient phenotyping in a case of accelerating hemolytic anemia to aid in donor RBC selection, the value of communicating with clinicians and the patient regarding the use of least-incompatible RBCs, and the importance of optimizing the patient’s clinical condition to avoid ischemia. In addition, it demonstrates the value of repeated attempts with IVIG treatment despite previous refractoriness to this treatment. *Immunohematology* 2004;20:177–183.

**Key Words:** autoimmune hemolytic anemia, multiple incompatible transfusions, IVIG therapy

Indications for RBC transfusion of patients with warm autoimmune hemolytic anemia (AIHA) include the risk for cardiac or cerebral ischemia. Transfusion of these patients may confer a risk of accelerated hemolysis due to stimulation of the autoimmune process, although this is a controversial notion (see reference 1 for a comprehensive review). However, there is also the risk for intravascular or extravascular hemolytic transfusion reactions due to the presence of alloantibodies in these often multitransfused patients. These alloantibodies may be very difficult to distinguish from the background of warm autoantibodies, and in cases of high-titer, low-avidity (HTLA) antibodies or antibodies to high-frequency antigens, the workup may be referred to a reference laboratory with access to rare cells and antisera.

It is not possible to define a critical Hb level below which transfusion should be administered during an exacerbation of hemolysis. The individual’s capacity to adjust to anemia depends not only on the level of Hb measured, but also on the rate of decline in Hb levels and the capacity for cardiac compensation. In cases with a background of cardiovascular disease, clinical symptoms and signs of cardiac ischemia guide the threshold for transfusion on an individual-case basis. The decision to transfuse such a patient thus depends on the clinical situation and extensive communication with the clinical staff.

**Materials and Methods**

ABO and Rh grouping, and screening for RBC antibodies, were performed by standard tube techniques. Similarly, RBC phenotyping was performed by standard tube techniques with commercially available typing sera. Antibody screening and panel cells were from multiple sources, including Immucor, Inc., Norcross, Georgia; Gamma Biologicals, Inc., Houston, Texas; and Ortho-Clinical Diagnostics, Raritan, New Jersey. All samples were tested with at least 11 different panel cells plus an autocontrol. DATs were performed using polyclonal anti-IgG, C3d (Biocline Ortho), rabbit anti-IgG (Immucor), and anti-C3b, C3d (Biocline, Ortho)-specific antisera.
Eluates were prepared by a rapid acid elution technique (Elu-Kit II, Gamma). Antibody enhancement was achieved using a PEG-based method (PEP GTI, Inc., Waukesha, WI) or treatment of RBCs with freeze-dried papain (Immucor). PEG autoadsorption was performed as previously described. Alloadsorption of serum was performed using untreated RBCs. Other methods performed by the American Red Cross National Reference Laboratory are as described in the case report.

Incompatible units were transfused using the in vivo crossmatch procedure, in which 30 to 50 mL of donor blood was slowly transfused into the patient, then the transfusion was temporarily stopped and a sample drawn from the patient and examined for visible evidence of hemolysis. In the absence of hemolysis, the remainder of the donor unit was transfused. A second sample was drawn from the patient at the end of the transfusion to look for visible hemolysis. This procedure was repeated with each unit transfused.

Case Report

A 66-year-old Caucasian woman, previously diagnosed with refractory AIHA, presented to the emergency department with increasingly severe hemolysis and angina on exertion, following about 3 weeks of increasing transfusion needs on an outpatient basis. Her Hct had dropped to 22.5% from 33% 4 days prior to admission. The Hb was 7.5 g/dL and the WBC count was 22,630/μL, with 88% neutrophils, 4% monocytes, 5% lymphocytes, 1% basophils, 2% metamyelocytes, and 6 nucleated RBCs/100 WBCs. The platelet count was 513,000/μL, and the reticulocyte count was > 30%. Total bilirubin was 4.5 mg/dL.

Past history

The patient was group O, D+ and had refractory AIHA diagnosed 6 years previously that required treatments including splenectomy, cyclophosphamide, rituximab, cyclosporine, and high-dose IVIG. A bone marrow biopsy performed at the initial presentation of disease showed no evidence of malignancy. For the previous 16 months she had not required blood transfusions and antibody screening tests had been negative for the previous 2 years. Additional medical history included coronary artery disease requiring bypass graft surgery more than 10 years previously, two minor strokes 2 years previously, hypertension, hyperlipidemia, and four uneventful pregnancies. On admission her medications included clonidine, diltiazem, prednisone at a dose of 15 mg/day, weekly erythropoietin injections, dietary iron supplementation, and opioid analgesics for chronic lower back and lower extremity pain. There had been no recent changes in her medication or any infectious prodrome prior to this most recent exacerbation of her hemolytic anemia.

Recent history

Three weeks prior to admission to the emergency department, the patient had an office visit for complaints of fatigue and mild angina. She had previously experienced chest pain when her Hct had fallen below 30%. At this time, the Hb was 10.6 g/dL and the Hct was 32.7%. On the basis of her mild angina symptoms, a RBC transfusion in an outpatient setting was ordered. The blood bank workup demonstrated strong microscopic reactivity with all antibody screening cells in the antiglobulin phase (but no reactivity at the immediate spin phase). The autocontrol was negative. Serum reactivity was positive to a dilution of 1:16 and could not be neutralized with plasma. A screen for cold agglutinins was negative. The use of papain-treated RBCs resulted in enhanced reactivity to 1+ with all antibody screening cells. No distinct specificity was apparent. An attempt to remove possible autorreactive antibodies by PEG autologous adsorption also generated strong to 2+ microscopic reactivity with all antibody screening cells. The serum also reacted with RBCs negative for Rh, Ch, Kp, Js, and U.

The DAT was negative and since the patient had not been transfused for more than 1 year, phenotyping was performed. The patient was negative for C, E, K, S, and Pl. Crossmatching with random RBC units showed strong microscopic reactivity. The interpretation of these serologic findings was that the antibodies detected were most consistent with nonneutralizable HTLA antibodies, but the possibility of antibodies to clinically significant, high-frequency antigens could not be excluded. Following discussions with the ordering physician, the patient, and her husband, the patient received the two least-incompatible K- units by in vivo crossmatch, without complications.

Before admission to the emergency department, the patient had received transfusions of six least-incompatible K- units by in vivo crossmatch on three occasions without complications, but at
increasingly shorter intervals between transfusions. Furthermore, the reactions to antibody screening cells and crossmatches had increased to macroscopic levels (1+). The DAT and autologous control cells remained negative. However, RBCs from the patient were noted to weakly autoagglutinate in the saline control. This effect could be removed with warm 37°C saline washing of the cells, suggesting the development of a cold agglutinin. Two allogeneic adsorptions using antibody screening cells, both with and without PEG enhancement, failed to completely remove reactivity that persisted against the cells used in the adsorption procedure. Antibodies against preservatives in the screening cells were ruled out by using multiply-washed screening cells. No correlation between the strength of reactivity and the age of crossmatched RBC units was identified. An extensive review of her medication list found no drugs reported to induce hemolytic anemia, although the patient’s husband did report a temporal association with the use of the anti-depressant mirtazapine in the past. However, a search of the literature showed no reported association.

**Extensive serologic workup**

Given the increasing strength of reactivity against allogeneic cells and the shortened RBC survival, there was a concern that the antibodies detected were against a clinically significant, high-frequency antigen. A more extensive serologic workup was requested from the American Red Cross (ARC) Blood Services—New England Region Reference Laboratory in Burlington, Vermont. Briefly, it was concluded that no specificity could be identified, but evidence of weak complement binding (microscopic) was detected using an enhanced DAT (5 minute incubation at 37°C), which suggested the presence of a warm autoantibody. It was further concluded that a nonneutralizable HTLA antibody was present. RBCs lacking the following high-frequency antigens were tested and found to be reactive with the patient’s serum: k, I, Js^b, Kp^a, Yt^b, U, Rg, Ch, Lan, AnW, Ge2, Ge3, Jr^b, Vel, PP11^b, Co^t, Jo^t, Gy^b, Hy, Yk^b, Kn^a, McC^b, McC^b, Cs^a, Lu^a, and JMH. A new specimen was then sent to the ARC National Reference Laboratory for Blood Group Serology in Philadelphia, Pennsylvania, for a more extensive evaluation.

Testing performed by the ARC National Reference Laboratory was similarly unable to identify an antibody specificity. The sample received by their laboratory was a later sample. The DAT was 1+ with polyspecific anti-human globulin, negative with monospecific anti-IgG, and 1+ with monospecific anti-C3d, and a saline control using warm saline 37°C washed RBCs was negative. Weak autoagglutination of the patient’s RBCs could be removed by warm 37°C saline washing. The patient’s serum reacted with all RBCs, including autologous cells tested by 37°C albumin, IgG antiglobulin test (AGT), and ficin IgG-AGT. RBCs treated with DTT were still reactive. Furthermore, DTT treatment of the patient’s serum changed reactivity from weak reactivity to 3+. The serum was reactive to a dilution of 1:256. Five adsorptions with r,r’, or r” RBCs did not remove this reactivity. However, after the fifth adsorption, the remaining reactivity was uniformly weakly positive against panel cells, suggesting that no strongly reactive antibodies existed against major RBC antigens. RBCs with the following phenotypes were found reactive at the albumin-IgG-AGT phase: JMH-, Di(b-), At(a-), Cs(a-), Yk(a-), Jk(a-b-), I-, PPI1^b-, D-, Rh^h, Cde/Cde, cdE/cdE, Lu(a-b-), Yt(a-), Co(a-), Lan-, Vel-, Kp(b-), McLeod, Sc-1, and J(o-). Finally, the antibody reactivity was assessed for clinical significance with the monocyte monolayer assay using group O, C-, E-, P1-, S-, K-, Fy(a-); group O, C-, E-, P1-, S-, K-, Fy(a-); and group O, C-, E-, P1-, S-, K-, Fy(a-) RBCs, with and without fresh complement, and using pooled monocytes from two donors. The reactivity of the patient’s serum was 21.5 percent to 34 percent reactive monocytes, consistent with a clinically significant antibody (normal range is 0-3%).

On the present admission to the emergency department, the patient had angina on exertion. There were no ischemic EKG changes and Troponin I was < 0.15 ng/mL (normal < 0.15, indeterminate 0.15-1.50, positive > 1.50 ng/mL). The blood bank workup at that point showed increased panel cell reactivity of 1 to 2+. Furthermore, the DAT was 1+ with broad-spectrum antiglobulin reagent, 1+ with IgG monospecific serum, and 1+ with anti-C3 monospecific serum. However, the saline control was now strongly positive microscopically, suggestive of autoagglutination. With warm 37°C saline washing of the patient’s RBCs, only the broad spectrum and anti-C3-specific reactions remained reactive at 1+. An eluate was performed and found to have nonspecific reactivity at the antiglobulin phase (weak to 1+ microscopic) for all RBCs tested. The autologous cell control at 4°C was also 1+. All crossmatched C+-, Fy(a-), K-, S- RBC units showed 1+ to 2+ reactivity.

At this point the risk for ischemic damage to the heart due to severe anemia was weighed against the
risk of exacerbating the autoimmune process underlying the hemolytic anemia with the transfusion of incompatible RBCs. Because at this point there were no clear clinical signs of a developing myocardial infarction, it was felt that transfusion should be held off, if possible, pending further testing results. However, the following night the patient started to complain of moderate chest pain at rest. Troponin I had increased to 0.29, Hb was 8.5 g/dL, and the Hct was 25.6%. The patient subsequently received two 1+ crossmatch-incompatible units by in vivo crossmatch without complications, following a bolus dose of 60 mg prednisone. She continued on 60 mg of prednisone per day. On day 3, Troponin I peaked at 0.44. Hb had decreased to 7.7 g/dL, the Hct was 23%, and the patient's chest pain persisted at the same level, necessitating further transfusions. Only 2+ incompatible units could be found and the patient received a unit of C\(^\text{w}-\), Fy (a\(^{-}\), K\(^{-}\), S\(^{-}\), P1\(^{-}\)- RBCs by in vivo procedure without complications.

On day 4, Hb was 9.3 g/dL and Hct was 27.8%. It was decided that the patient would undergo cardiac catheterization and coronary artery stents were placed, with immediate relief of symptoms. On day 5, Hb had decreased to 8.0 g/dL, the Hct was 23.9%, and total bilirubin had increased to 5.8 mg/dL, confirming increasing hemolytic activity. Reactivity of crossmatched phenotype matched units (C\(^\text{w}-\), Fy a\(^{-}\), K\(^{-}\), S\(^{-}\), P1\(^{-}\)) was generally increased to 1+ to 3+; however, two microscopically reactive units were found. Over the following 5 days, the patient continued to receive least-incompatible phenotype-matched units without immediate complications or evidence of intravascular hemolysis. However, there was an increase in the total bilirubin (7.7 mg/dL on day 6), an increase in the strength of reactivity against crossmatched units, and increasing frequency of transfusion requirements (two RBC units every 2–3 days). Reactivity to crossmatched units continued to increase to up to 4+ on day 7.

Following 10 days of high-dose prednisone treatment without discernible improvement, high dose IVIG therapy was attempted with a one-time dose of 1g/kg body weight. Three days later, Hb had reached 10.6 g/dL, Hct was 31.8%, and total bilirubin was 2.1 mg/dL. The patient was no longer in need of transfusions and could be transferred to a rehabilitation unit, where she continued to maintain Hb values around 11 g/dL, and Hct well above 30% (range of 33%–37%). A bone marrow biopsy was scheduled and then canceled due to the patient's dramatic improvement. The patient's medication at time of transfer included prednisone 60 mg/day, erythropoietin, folic acid, and clopidogrel.

**Discussion**

Differentiation between a preexisting warm-reactive autoantibody and an antibody against a high-frequency antigen appearing after a transfusion is difficult. However, critical results in this patient's evaluation were a negative DAT and a negative autologous control. However, with continued transfusion, positive results with gradually increasing strength were seen in these tests. With these findings, warm-reactive autoantibodies were considered less likely, but could not be completely excluded, and more thought was given to the possibility of a HTLA antibody or an antibody against a high-frequency antigen. A HTLA antibody was initially favored given the weak reactivity that persisted to dilutions of 1:16. However, concern for a clinically significant antibody, especially against a high-frequency antigen, began to grow as the patient began to require more RBC transfusions at shorter time intervals, and the reactions obtained with panel cells and crossmatches increased to macroscopic reactivity, considered unusual for a HTLA antibody. Although the DAT and autologous cell control were initially negative, the patient likely had a chronic immune hemolytic anemia that was previously kept under control with erythropoietin and prednisone therapy. Therefore, the confluence of an autoimmune hemolytic process, HTLA antibody, and possible antibody against a high-frequency antigen rendered the decision to transfuse crossmatch-incompatible RBCs that much more difficult.

To aid in the selection of RBC units for transfusion of patients with unidentified specificities, phenotyping for common antigens of the Rh, K, Jk, Fy, and MNS systems should be performed on the patient's RBCs at the first opportunity prior to RBC transfusion. In this case, the patient had not been transfused in more than a year and presented with a negative DAT. While difficult, even in patients who have been transfused over the past 3 months, a phenotype can be obtained based on either molecular or serologic techniques. With the knowledge of which major antigens are lacking in the patient, decisions can then be made in identifying appropriately phenotype-matched units that would be least likely to generate a hemolytic transfusion reaction and would survive longer in the patient's circulation.
When searching for RBC units that are antigen negative, the difficulties increase as multiple antigens are required to be simultaneously absent. The need for using only partially matched or incompatible units arises and choices must be made as to which antigens to ignore in screening for antigen-negative RBC units to transfuse. The degree of immunogenicity of each antigen must then be taken into account, as well as the severity of the possible consequences of transfusing each antigen. Certain combinations of antigen-negative RBC units are more easily found than others and must also be taken into account in a search. Furthermore, results of adsorption studies may indicate the absence of any antibodies against major RBC antigens and help in guiding which antigens could be more comfortably ignored. In this patient's situation, adsorptions performed at the reference laboratory failed to completely adsorb all antibodies but reduced the reactivity to only microscopic reactivity with all cells tested. The inference from this finding was that while no statement could be made regarding reactivity against high-frequency antigens, no strongly reacting antibodies against major RBC antigens were identified. However, adsorptions would need to be performed at 3 to 4 day intervals to rule out their emergence if the patient continued to be transfused with units positive for antigens that the patient lacked.

In the absence of the ability to identify antibody specificities and compatible RBC units by routinely used serologic tube-based methods, alternative methods exist for determining if an antibody may be clinically relevant. The monocyte monolayer assay quantifies rosetting or phagocytosis of antibody-sensitized cells by monocytes. In this case it indicated that the antibodies in the patient's serum had clinical significance, i.e., would be likely to cause decreased survival of transfused RBCs. In this particular case, the monocyte monolayer assay might have been more useful if donor cell phenotypes were identified that predicted increased RBC survival. Other reported methods that have been used include $^{51}$Cr labeling of RBCs and flow cytometry. The results of such assays may help evaluate the current immunization status and help reveal alloimmunization by comparing the survival of autologous RBCs with allogeneic RBCs. However, because an antibody may develop at any time during a period of repeated transfusions, the results cannot be used to exclude the existence of clinically significant alloantibodies in the clinical setting beyond the point of the next transfusion.

An alternative option that may be available in the future is the use of blood substitutes. Hemoglobin-based blood substitutes are currently in phase III clinical trials for various indications and have been used successfully in at least one instance for AIHA. The most effective use of hemoglobin-based blood substitutes would be to temporarily stabilize the patient until difficult-to-find or rare donor blood units could be obtained or until more aggressive immunosuppressive regimens have had a chance to take effect. In both instances however, there would be a prolonged requirement for the use of blood substitutes and the introduction of free hemoglobin into the patient's circulation would interfere with the visual assessment of hemolysis.

The balance between the risks of inadequate oxygenation and subsequent ischemic damage on the one hand, and the risks of transfusing incompatible RBCs on the other hand, represents a clinical challenge. In situations where there is a critical need to optimize oxygen delivery by increasing RBC volume but for which no compatible units can be found, transfusion by in vivo crossmatch is unavoidable. In these instances, such as in this case, close communication with the clinicians is essential to give adequate input into the decision-making process. In this context it should be emphasized that the in vivo crossmatch has a limited value in predicting survival of the transfused RBCs, since one is only looking for acute gross intravascular hemolysis during the transfusion. Therefore, a negative in vivo crossmatch result only indicates that the likelihood of acute intravascular hemolysis and its attendant morbidity and mortality is lessened. Furthermore, in situations where the in vivo crossmatch procedure has been repeated multiple times with negative results, continuous awareness of the increased risks of transfusing otherwise incompatible RBCs is imperative for both the physicians who order the transfusions and the nurses who administer them. Finally, it is important to communicate with the clinicians and the patient the exact nature of "least-incompatible blood" and its increased risks, to address any concerns regarding the in vivo crossmatch procedure, and to further explain why no compatible blood could be found.

Apart from identifying least-incompatible RBC units for transfusion, therapeutic options for patients with AIHA are generally aimed at reducing the clearance of RBCs and reducing the production of antibodies. Corticosteroids usually constitute the first
line of treatment. Splenectomy is used when the patient is refractory to treatment with corticosteroids. Immunosuppressive therapy, such as cyclophosphamide and azathioprine and short-term use of cyclosporine, is sometimes successful in inducing remission in these otherwise refractory patients. The use of rituximab in AIHA, a monoclonal antibody that causes specific B-cell depletion by targeting the B-cell CD20 antigen, has been reported.\textsuperscript{15-18} For patients who are critically anemic and who are refractory to transfusion, plasma exchange may be considered, but it is highly inefficient at the removal of IgG antibodies, and better suited for patients with hemolysis due to IgM antibodies.\textsuperscript{19}

The immunomodulatory action of IVIG therapy, although not well understood, has been used successfully for a myriad of autoimmune disorders.\textsuperscript{20} Although unresponsive to this therapy in the past, our patient responded promptly to administration of IVIG. There are substantial differences in IVIG, based on differences in purification and chemical stabilization by manufacturers as well as lot-to-lot variations due to variation in the donor pool, even with the same manufacturing process. Such differences have been noted in the use of IVIG as an immunomodulatory therapy in solid organ transplantation\textsuperscript{21} and may explain the different responses to IVIG in this patient.

In conclusion, this case illustrates the management of accelerating AIHA with critical levels of Hb in association with threatened cardiac ischemia and possible alloantibodies. It demonstrates the usefulness of performing phenotyping in these patients at first opportunity and the necessity to thoroughly work up the antibody reactivity in the presence of a negative autologous cell control. Furthermore, it exemplifies the importance of optimization of clinical conditions, in this case performing cardiac catheterization and stent placement to maximize oxygen delivery to the heart. In addition, it shows that IVIG may be useful and should be attempted in these patients even if prior treatments with IVIG have been unsuccessful.

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