Life-Threatening Autoimmune Hemolytic Anemia Treated with Manual Whole Blood Exchange with Rapid Clinical Improvement

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Abstract

Severe autoimmune hemolytic anemia, with high titer panagglutinins, can present with severe intra-and extravascular hemolysis, complicated by renal failure, volume overload, hypertriglyceridemia, pancreatitis and multorgan failure. We present a case of life-threatening warm autoimmune hemolytic anemia in a 19 year old male, refractory to steroids and splenectomy, with ongoing severe hemolysis, in vivo and in vitro autoagglutination, worsening hypoxia, methemoglobinemia, renal insufficiency, and hypertriglyceridemia. The patient underwent a single manual whole blood exchange with reconstituted whole blood (50% final hematocrit). The post-exchange hemoglobin was 7.7 gm/dL, with complete resolution of RBC autoagglutination, increased platelet count (20%) and marked decreases in plasma free hemoglobin (43%), methemoglobin (9%), percent FiO₂ (40%), creatinine (30%), triglycerides (66%), bilirubin (56%) and autoantibody titer (1000 to 256). The patient was extubated shortly after WBEx with minimal hemolysis and RBC transfusion support over the next 2 weeks.

Following a single WBex, the patient defervesced with rapid improvement in oxygenation, renal function and hemolysis.

Materials and Methods

Serology

Samples for patient testing were collected in EDTA per routine. Spontaneous agglutination was resolved by washing patient's red cells with prewarmed saline at 37°C four times [3]. ABO and Rh (D, C, E) RBC typing were performed with commercial reagents (Ortho Clinical Diagnostics, Raritan, NJ). An extended RBC genotype was performed by the University of Michigan Molecular Pathology Laboratory using the Red Cell EZ type reagents (Hologics/GenProbe, San Diego, CA). The patient's predicted RBC phenotype was rr, kk, Jk (a-b+), Fy (a+b+), MNs.

Direct antiglobulin testing (DAT) was performed by tube method using commercial anti-human IgG, anti-human C3 and polyspecific (IgG, C3) reagents: 6% albumin was always included as an inert control. IgG isotype analysis was not performed. RBC eluates were prepared with EDTA-glycine-acid (EGTM™ kit, Immucor/Gamma, Norcross, GA). Eluates were tested against untreated and ficin-treated RBC using polyethylene glycol enhancement (GammaPeG™, Immucor/Gamma).

Plasma reactivity was examined at immediate spin, room temperature; 60 minute, 37°C incubation and the IAT against

Keywords: Blood exchange, Autoimmune hemolytic anemia

Abbreviations: AIHA: Autoimmune Hemolytic Anemia; DAT: Direct Antiglobulin Test; Hgb: Hemoglobin; IAT: Indirect Antiglobulin Test; 2-ME: 2-Mercaptoethanol; Prbc: Packed RBC Units; SCID: Severe Combined Immunodeficiency Disorder; WAIHA: Warm Autoimmune Hemolytic Anemia; WB: Whole Blood; Wbex: Whole Blood Exchange

Introduction

Autoimmune hemolytic anemia (AIHA) is a relatively rare disorder with an incidence of 1-3 per 100,000 [1]. Warm AIHA (WAIHA) is typically due to IgG autoantibodies with optimal reactivity at 37°C and account for the vast majority of AIHA cases (80-90%) [1,2]. Clinical presentation can range from an asymptomatic, compensated hemolytic anemia to acute life-threatening hemolysis. Hemolysis is typically extravascular; however, complement-mediated intravascular hemolysis can be seen at high levels of RBC bound antibody (>1000 molecules IgG), in rare IgM WAIHA and in mixed AIHA characterized by IgM and IgG autoantibodies [2-5]. Although death from WAIHA is considered rare, mortality rates of 4-11% have been reported in children [6,7]. Mortality is particularly high in mixed and WAIHA due to IgM autoantibodies possessing high thermal amplitudes [3-5].

The standard treatment for WAIHA is high dose steroids, which are effective in 70-80% of patient within 2 weeks of starting therapy [1,2]. Steroid-refractoriness is observed in approximately 20% of patients, who are typically treated by either splenectomy and/or a trial of rituximab. Alternative salvage therapies for resistant WAIHA, as single agents or in combination, are danazol, IVIG, alemtuzumab, cyclophosphamide, azathioprine, cyclosporine, mycophenolate mofetil, and vincristine-loaded platelets [1,8-10]. Plasmapheresis has also been tried in severe AIHA as adjunct therapy, usually in combination with multi-agent immunosuppressive regimens [11-13].

There are a handful of reports utilizing whole blood exchange (WBEx) in severe AIHA. In general, WBEx has been used to avoid volume overload in patients requiring massive RBC transfusion support or to treat in vivo hemagglutination [4,14-16]. We report the use of manual WBEx in a young man with life-threatening hemolysis, RBC autoagglutination, respiratory failure, methemoglobinemia, impending acute renal failure and hemolysis-associated hypertriglyceridemia.

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unmodified and ficin-treated RBC [17]. Indirect antiglobulin testing (IAT) was performed by tube and gel method using anti-IgG and anti-polyispecific reagents. To remove autoantibody, plasma was adsorbed (x4) against group O, rr, lk (a-) RBC treated with ZZAP [17,18]. To exclude IgM autoantibody, patient plasma was incubated with PBS or 2-mercaptoethanol (2-ME) for 2 hours at 37°C, followed by overnight dialysis as described [17]. Autoantibody titration was performed by the IAT tube method (60; 37°C, anti-IgG) using two-fold dilutions of plasma diluted in normal saline and tested against group O, rr, unmodified RBC. The autoantibody titer was the last dilution showing 1+ reactivity [17]. The autoantibody score was calculated as described by Marsh [19].

Whole blood exchange

Reconstituted whole blood (WB) was prepared from group A plasma and pre-storage leukoreduced, group A, Rh-negative packed red blood cells (pRBC) in Adsol anticoagulant. PRBC units were less than 15 days of age and matched for Rh and K antigens (C-, E-, K-). PRBC were centrifuged and resuspended in plasma to a 50% final hematocrit (Hct) [20]. Because of its short outdate [21], WB was prepared and dispensed one unit at a time. WB units were crossmatched against unasorbed patient plasma and labeled ‘crossmatch-incompatible’. For WBEx, a right internal jugular cordis central line was placed for venous access. WB was removed with 50 mL syringes over a period of 10-15 minutes, followed by infusion of WB.

Case Description

The patient was a morbidly obese (weight 121 kg, BMI 40.4), 19 year old white male who presented to his local emergency room with a 5 day history of fatigue, malaise, anorexia, right flank pain, dark urine and new nausea and vomiting. His history was unremarkable except for intermittent headaches during the last 2 months. He was not taking prescription medication and had no prior significant past medical history. Laboratory studies showed anemia and hyperbilirubinemia. The patient was subsequently transferred to a larger facility for further evaluation and treatment.

On admission to the second facility, the patient was febrile and jaundiced, with a hemoglobin (Hgb) 7.7 gm/dl, an elevated LDH (1625 IU/L), low haptoglobin (<10 mg/dL) and indirect hyperbilirubinemia (total bilirubin=10.4 mg/dl, indirect bilirubin=10 mg/dL). The patient typed as group A, Rh-negative with a positive antibody screen due to a strong (4+) panagglutinin. His DAT was reportedly positive with both anti-IgG and anti-C3. A CT scan showed splenomegaly and bilateral pulmonary lower lobe opacities with consolidation. No lymphadenopathy or masses were noted by CT or physical exam. The patient was admitted and received 4 units of group A pRBC with no increase in Hgb. Within 12 hours of admission, the patient was transferred to the ICU with tachypnea (RR=28), tachycardia (HR=129), hypotension (SBP=80), hypoxia (Percent O2 saturation=80% on 6L O2) and new nonspecific ST changes by EKG. The patient was intubated and transferred to a university medical center the morning after admission.

On arrival at the university hospital, the patient was febrile (T=39.1°C), tachycardic (HR=112 bpm), normotensive (BP=140/55), intubated (%FiO2=40%, O2 saturation=95-99%) with jaundice and palpable splenomegaly. He had worsening anemia (Hgb=5.7 gm/L) with elevated reticulocytes (0.25%) but normal RBC indices, platelet (164x10^9/L) and WBC counts (11.7x10^3/L). A peripheral blood smear showed some rouleaux but no increase in spherocytes or schistocytes. A WBC differential showed an absolute and relative neutrophilia (79%) with toxic granulation and dohle bodies. Chemistry studies showed absent haptoglobin (<10 mg/dL); elevated plasma free Hgb (214 mg/dL), LDH (2686 IU/L), AST (675 IU/L), indirect bilirubin (total bilirubin=11.6 mg/dL, indirect=10.2 mg/dL [88% total]), and hemoglobinuria. (79%) with toxic granulation and dohle bodies. Chemistry studies showed absent haptoglobin (<10 mg/dL); elevated plasma free Hgb (214 mg/dL), LDH (2686 IU/L), AST (675 IU/L), indirect bilirubin (total bilirubin=11.6 mg/dL, indirect=10.2 mg/dL [88% total]), and Coagulation studies showed a normal PT, aPTT, d-dimer and elevated fibrinogen (550 mg/dL). The patient’s admission renal function showed a prerenal azotemia with elevated BUN (49 mg/dL), slightly elevated creatinine (1.5 mg/L) and abundant granular casts (26-50/hpf) on urine microscopy. An admission lipid panel showed mildly elevated triglycerides (248 mg/dL; cholesterol, 177 mg/dL). An abdominal ultrasound showed splenomegaly. A chest X-Ray showed no acute disease.

Diagnostic tests were unrevealing. The patient was negative for cryoglobulins, cold agglutinins, ANA, ANCA, MPO, PR3, and anti-phospholipid antibodies. Serum IgG, IgM, IgA and C3 levels were normal; however, the C4 was low (0.2 mg/dL). A serum electrophoresis showed an acute phase pattern only: No C-reactive protein or ESR was performed. Several sets of blood, urine and sputum cultures were negative. Serology and PCR studies showed no evidence of acute infection with CMV, EBV, HIV, HBV or HCV. Thrombotic thrombocytopenia purpura (ADAMST13 activity=48%) and paroxysmal nocturnal hemoglobinuria (no detectable CD14+, FLAER-monocytes) were excluded. The patient did have an elevated urine copper (1035 µg/24 hours; normal range <55 µg) suggestive of Wilson’s disease; however, the patient had repeatedly normal ceruloplasmin
levels (x2), no ATP7B gene mutations, and no Kayser-Fleisher rings on slit lamp exam.

Blood bank testing was complicated by the presence of a strong autoantibody with both RBC autoagglutination and spontaneous agglutination [3], even after washing patient RBC eight times with normal saline. Agglutination was resolved after repeatedly washing RBC with warm saline [3,17]. The DAT was positive with polyclonal (4+) and IgG (4+) but not complement. His plasma contained a strong panreactive antibody with anti-IgG at 37°C by gel and tube methods. Reactivity was enhanced with ficin-treated RBC, but not by incubation at cooler temperatures. An eluate was positive with all cells tested in the IAT. Plasma reactivity was not affected by treatment with 2-ME. No alloantibodies were identified after testing adsorbed plasma.

Upon admission, the patient was treated with methylprednisolone (6 mg/kg), pRBC transfusions, folic acid, antibiotics (azithromycin, vancomycin) and hydration to prevent pigment nephropathy. Over the course of 3 days, the patient was transfused with 14 units pRBC with no sustained increase in Hgb (Figure 1). On day 4, the patient underwent an open splenectomy with minimal intraoperative blood use (1 pRBC). The spleen was enlarged, measuring 15 cm and weighing 730 gm. White pulp atrophy consistent with steroids, extramedullary hematopoiesis and florid hemophagocytosis were noted on histology.

Following splenectomy, the patient had continuing hemolysis accompanied by worsening spontaneous hemagglutination (37°C) and RBC microaggregates on peripheral blood smears (Figure 1). In addition, the patient's DAT was now positive for complement (IgG=4+, C3=2+). The patient's clinical condition continued to decline with increasing renal insufficiency (Figure 2C, peak creatinine=2.6 mg/dL, BUN=100-108), fever (Tmax=40°C) and new rhabdomyolysis (creatinine kinase=3490 IU/dL). He developed increasing oxygenation requirements (FiO₂=80-100%, O₂ saturation=80-90%), concurrent with rising methemoglobinemia (peak 14.7%), cyanosis and lactate (peak lactate=7.2 mEq/L). There was a ten-fold increase in triglycerides (248 to 2653 mg/dL; cholesterol=177 mg/dL), felt to be secondary to massive hemolysis and systemic inflammation [22]. A serum amylase and lipase were not performed. The patient was placed on esmolol for new hypertension (BP 200s/90-107). A trial of rituximab on day 6 was suspended after the patient had a reaction to the test dose (100 mg).

On day 6, the hospital transfusion medicine service was consulted regarding possible plasmapheresis as prophylaxis against acute renal failure and pancreatitis by pigment nephropathy and hypertriglyceridemia, respectively [23-25]. In addition, it was hoped that plasmapheresis might slow the rate of hemolysis. After review, it was felt that the patient was not sufficiently stable to undergo apheresis. Furthermore, the severity of hemagglutination at 37°C raised technical concerns regarding the ability to perform the procedure. Specifically, there was a significant risk of RBC aggregation, procedure-related hemolysis, and circuit occlusion with circuit loss by exposure of patient blood to the centrifugal shear forces and cooler temperatures within the extracorporeal circuit.

Instead of plasmapheresis, it was decided to proceed with a manual WBEx using reconstituted WB. Unlike apheresis, manual WBEx had minimal clinical or procedural risks, while providing RBC transfusion support. In addition, WBEx could remove triglycerides and plasma free Hgb as well as methemoglobin and circulating RBC microagglutinates. Given the severity of the patient’s hemolysis, we opted to reconstitute pRBC to a standard final Hct of 50% used in neonatal WBEx. His estimated total blood volume, corrected for BMI, was 4800 mL. The patient received 12 WB units (4320 mL, 89% total blood volume) over 20 hours with no adverse events. His post-procedure Hgb=7.7 gm/dL and was accompanied by a resolution of RBC autoagglutination (Figure 3A and 3B). WBEx successfully decreased methemoglobin (Figure 2B),
plasma free Hgb (43%, Figure 1A), triglycerides (66%, 2653 to 903 mg/dL), and total bilirubin (56%, 13.6 to 5.9 mg/dL).

Following WBEx, the patient became afebrile. There was a significant improvement in hemolysis as evidenced by decreased pRBC transfusion frequency (Figure 1) and falling plasma free Hgb with clearance of serum (Figure 1A and 3C). The resolution in hemolysis was accompanied by improvements in renal function and platelet count (Figure 2C and 2D). The patient’s percent FiO2 requirements dropped rapidly with extubation early on Day 11 (Figure 2B). Parallel testing of his pre- and post-WBEx samples showed a significant decrease in autoantibody titer (1000 to 256) and Marsh score (108 to 82).

The patient was successfully rechallenged with rituximab on day 10 followed by 3 additional weekly doses. He was transitioned from methylprednisolone to oral prednisone on day 14. Following WBEx, the patient required only 5 more pRBC transfusions over the next 11 days, with his last transfusion on day 19 (Figure 1). His autoantibody levels continued to fall over the next two weeks (IAT=2+, neat plasma; day 25). He was discharged from the hospital on day 43. Testing 18 months later showed a negative DAT and antibody screen.

Discussion

With rare exceptions, WAIHA is due to IgG autoantibodies, with 50-75% of cases also positive for complement [2,7]. Patients can present with a severe hemolytic anemia requiring aggressive medical treatment and transfusion support. Due to their severe anemia, these patients are at risk for volume overload and pulmonary edema from frequent transfusions, an expanded plasma volume, and hydration to prevent pigment nephropathy. Additional complications in these patients include renal failure, disseminated intravascular coagulation and multiorgan failure from massive, ongoing intravascular release of free Hgb and RBC stroma [1,25]. Death in WAIHA is usually the result of cardiovascular collapse, pulmonary emboli or infection [1,4,5].

We present a case of life-threatening, refractory WAIHA that was successfully treated with a single manual WBEx with rapid and dramatic clinical improvement. Manual WBEx was chosen over plasmapheresis in our patient since it presented minimal risk, provided RBC transfusion support, and permitted concurrent plasma and RBC exchange. A single manual WBEx led to acceptable decreases in plasma free Hgb, triglycerides, and bilirubin. WBEx was also highly effective.
in reducing methemoglobin, with parallel improvement in oxygenation and ventilator settings. Methemoglobin levels as low as 8-10% is associated with low oxygenation saturation and hypoxia due to a loss of reversible O$_2$ binding and left-shift in the O$_2$ dissociation curve [26-29].

Most surprising, however, was the resolution of RBC autoagglutination, C3 positivity and hemolysis shortly following WBEx. The patient showed continuing clearance of free Hgb for several days after the procedure (Figure 1 and 3C). More importantly, his transfusion requirements dropped from 31 units pre-WBEx to only 5 RBC units post-WBEx. Two potential and complementary mechanisms for these findings are in vivo adsorption of free autoantibody and removal of heavily-coated, sensitized RBC. During the course of WBEx, the patient slowly received approximately 2100 ml of PRBC, which provided time and substrate for autoantibody adsorption. As a result, WBEx may have lowered the amount of RBC-bound IgG below a critical threshold necessary to support autoagglutination and complement activation.

WBEx may also have removed plasma factor(s) capable of exacerbating hemolysis and premature eryptosis analogous to that described in hyperhemolysis syndrome [30]. One possible candidate is methemoglobin, a potent oxidant, which can precipitate hemolysis by intra- and extra-erythrocytic mechanisms. Intracellular methemoglobin associates with Band 3 and cytoskeleton, leading to Heinz body formation, oxidative damage and altered RBC deformability [29]. More recent studies show that intracellular methemoglobin also leads to oxidative damage to the RBC membrane with increases in RBC fragility, aggregability and phosphatidylserine exposure. Because methemoglobin is rapidly generated from free Hgb following RBC lysis, it is hypothesized that free methemoglobin may synergize and amplify ongoing hemolysis in malaria and other hemolytic disorders [31,32].

There are only a few published reports of WBEx in AIHA (Table 1). There are two prior reports in classic WAIHA, although the methods and replacement fluids used differ from classic manual WBEx. Garelli and colleagues described a single WBEx in a 40 year old man with severe post-infectious WAIHA [14]. This patient initially underwent a 2 unit manual RBC exchange, followed by automated plasmapheresis using a cocktail of WB, plasma and colloid for replacement fluids. Like our patient, the patient had rapid improvement within 24 hours. A second case was reported in a recent abstract and describes the use of concurrent 2L WB phlebotomy and reconstituted RBC transfusion using two synchronized infusion pumps in a woman with severe refractory WAIHA and chronic lymphocytic leukemia (CLL) [15]. Unlike our case, RBC was often reconstituted with saline, albumin, plasmalyte or plasma. The patient was successfully supported over 9 days with stabilization and rise in Hgb and a decrease in autoantibody levels (IAT, 2+ to w+).

There are also three reports using WBEx in IgM-mediated AIHA (Table 1). John Hopkins reported the use of multiple WBEx in two pediatric cases with IgM-mediated WAIHA [4,5]. One child was an 11 month old girl with severe combined immunoencephalopathy (SCID) who underwent a related haploidentical bone marrow transplantation complicated by a severe WAIHA on day 2 [5]. The second patient was a 9 year old with Evan's syndrome [4]. Both patients received aggressive immunosuppression and twice daily WBEx with ongoing hemolysis, hemagglutination, multiorgan injury and death within weeks. A third case was reported by Schnitzer, who reported a fatal case of an anti-Pr cold AIHA secondary to Mycoplasma infection [16]. This patient received multiple automated WBEx totaling 41 units of blood without improvement and death 5 days after presentation. The inability of WBEx and aggressive immunosuppression to moderate hemolysis in these three cases is testimony to the unique severity of AIHA due to warm-acting, high-affinity IgM autoantibodies [2,3,33,34].

The severity of our patient’s presentation did raise concerns of a warm IgM component to his AIHA. Like an IgM-WAIHA, the patient presented with an unusually severe, unresponsive AIHA, with both extravascular and intra-vascular hemolysis, worsening hypoxia and impaired peripheral circulation with mottling and cyanosis. However, the patient’s serologic workup did not fit a classic cold or warm IgM autoantibody. The patient’s antibody was optimally reactive at 37°C and anti-IgG, with no enhancement at room temperature. In addition, the DAT with warm washed RBC was overwhelming IgG (++) whereas IgM-mediated AIHA is characterized by strong complement binding [2,3]. The transient C3 positivity observed immediately post-splenectomy may reflect the critical role of splenic macrophages in clearing C3-coated RBC [2]. Finally, there was no change in antibody strength or reactivity after treatment of plasma with 2-ME, thus excluding an IgM autoantibody [3,17].

There are anecdotal case reports of plasmapheresis in patients with severe refractory AIHA. Although a decrease in autoantibody can often be documented, clinical efficacy is highly variable with many studies reporting no apparent decrease in hemolysis or transfusion frequency [11,35]. Plasmapheresis may be particularly ineffective in classic IgG-mediated WAIHA since IgG has limited intravascular distribution (40-45%), finite removal by apheresis and rapid post-procedure rebound [36]. In patients with severe WAIHA, it may be extremely difficult to significantly reduce autoantibody levels due to high titers and biosynthetic rates. Not surprisingly, reports of plasmapheresis successfully moderating hemolysis involved multiple apheresis procedures in conjunction with aggressive immunosuppression [12,13]. Furthermore, the circulating free autoantibody present in plasma represents only a small fraction of the total intravascular autoantibody—most of which has already bound and sensitized RBC. At present, plasmapheresis for WAIHA is considered a category III indication by the American Society of Apheresis [37].

In summary, we describe the successful use of manual WBEx in life-threatening WAIHA. A single WBEx was well tolerated; with acceptable decreases in plasma free Hgb, triglycerides, methemoglobin, and resolution of RBC autoagglutination. The improved oxygenation and perfusion observed post-WBEx may correspond to correction of the O$_2$ dissociation curve, blood viscosity and peripheral vasodilatation due to methemoglobin, RBC agglutinates and free Hgb, respectively [29]. WBEx may also have quieted hemolysis through removal of heavily sensitized RBC (IgG+, C3+), in vivo adsorption of autoantibody and circulating methemoglobin. Advantages of manual WBEx over plasmapheresis are safety, concurrent removal of RBC and plasma; ability to provide aggressive isovolemic RBC transfusion support; and lack of expensive equipment and skilled personnel. Disadvantages are the time and labor, especially in adults with large blood volumes. Based on the literature, WBEx may be more beneficial in IgG-mediated WAIHA.

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