Prolonged in vitro hemolysis of EDTA-anticoagulated blood after a delayed hemolytic transfusion reaction

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BACKGROUND: A novel case where in vitro hemolysis was observed in plasma, but not in serum samples, obtained after the onset of a severe delayed hemolytic transfusion reaction is presented.

CASE REPORT: A 54-year-old woman received 2 units of blood during an orthopedic procedure. She had received transfusion 30 years earlier, and testing before transfusion revealed no alloantibodies. The patient returned 12 days after the transfusion with a Hb level of 54 g per L due to a severe delayed hemolytic reaction caused by anti-K. The plasma and serum samples were grossly hemolysed on Day 12. On Day 14, the serum samples showed no evidence of hemolysis; however, the EDTA sample remained grossly hemolysed. This discrepancy was not identified until Day 19. Due to concerns of ongoing apparent severe hemolysis, the patient was unnecessarily treated with IVIG and corticosteroids. The in vitro hemolysis was still present at 75 days, despite complete normalization of her Hb, bilirubin, and LDH levels. The phenomenon had resolved by 125 days.

CONCLUSION: This in vitro artifact has not been previously reported and the mechanism remains unclear. Both plasma and serum samples should be observed for hemolysis when evaluating a patient with a severe delayed hemolytic transfusion reaction.

ABBREVIATION: DHTR = delayed hemolytic transfusion reaction.

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We report a novel case where the in vitro hemolysis of plasma, but not of serum, was observed after a severe delayed hemolytic reaction (DHTR). This in vitro phenomenon has not been previously reported and has implications for the evaluation and treatment of DHTR.

CASE REPORT

A nulliparous 54-year-old woman received 2 RBC units prepared by immediate-spin crossmatch after a negative antibody screen that was performed by a column agglutination technique (ID-Micro Typing System, Micro Typing Systems Inc., Pompano Beach, FL). Her transfusions followed a debridement procedure for a localized soft tissue infection over her knee-joint prosthesis. At the time of surgery (Day 0) she began taking cefazolin and rifampin (Fig. 1). The patient had received a transfusion 30 years earlier.

The patient was admitted on Day 12 with pallor, jaundice, fatigue, and diarrhea. Her Hb level was 54 g per L, and her total bilirubin was markedly elevated (Fig. 1). The blood film demonstrated features of brisk hemolysis including marked spherocytosis, polychromasia, and leukoerythroblastosis. The initial plasma and serum samples from the patient were grossly hemolysed, and serum haptoglobin was not detectable. Antibody investigations demonstrated anti-K.

We confirmed that the patient's antibody screen and DAT were negative before Day 0 by using both tube and column agglutination methods with enzyme-treated and untreated RBCs. The patient's phenotype was K–, and one of the two units of RBCs transfused was K+. Twelve days after the incompatible transfusion, the DAT was positive for IgG (2^+) and C3b/d (4^+). Anti-K was detected in the eluate. At no time (Days 12, 14, and 23) were K+ RBCs detected by agglutination methods. The DAT was positive for both IgG and C3b/d when the patient was tested at Days 35, 75, and 125. No Donath-Landsteiner antibodies were detected when tested at Day 35. The sucrose lysis test was negative, and the expression of CD55 and CD59 on the patient's RBCs examined by flow cytometry (REDQUANT CD55/CD59 Kit, Biocytex, Marseille, France) was found to be normal. Four units of K– blood were transfused on Day 12. Cefazolin and rifampin

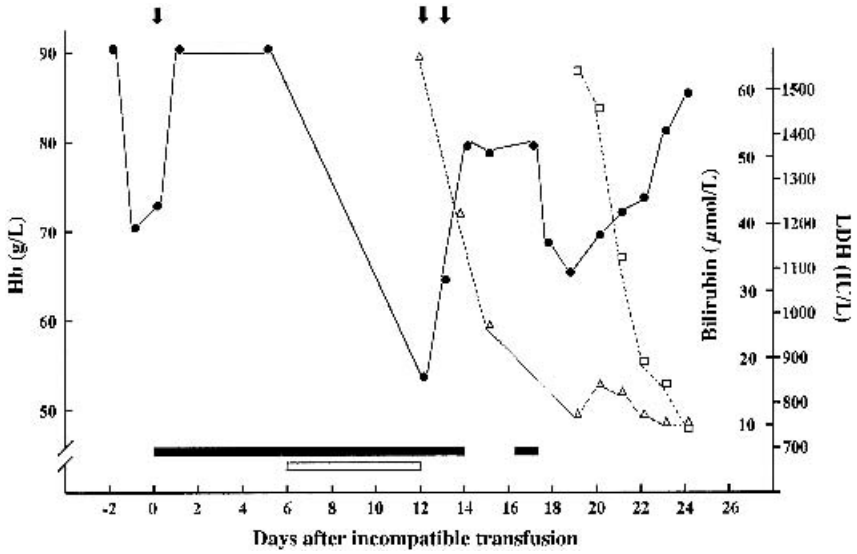

Fig. 1. The patient's clinical course. Each solid arrow represents a transfusion of 2 units of RBCs. The duration of treatment with cefazolin and rifampin are represented by the solid and open bars, respectively. Values for the Hb (g/L; ●), bilirubin (μ*M*/L; △), and LDH (IU/L; □) are shown for the first 24 days.

Fig. 2. Samples of EDTA-anticoagulated plasma, serum (gel-separator tube), and heparin-anticoagulated plasma taken from the patient at Day 24.

were discontinued and replaced by vancomycin on Day 14, as drug-induced hemolysis had not yet been excluded. The patient's Hb level stabilized at 80 g per L from Day 14 to 17; however, because her plasma samples remained grossly hemolysed, we transferred the patient's care to the hematology service and she was treated with IVIG and steroids for apparent ongoing intravascular hemolysis. The serum tubes drawn for biochemistry did not show any evidence of hemolysis after Day 14. This information was unavailable to us because these samples were processed at another hospital campus. We became aware of this discrepancy on Day 19 when the blood bank requested both serum and plasma samples.

Figure 2 demonstrates the marked degree of hemolysis in the EDTA plasma samples and a milder hemolysis in the heparin-anticoagulated samples (plastic, Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Hemolysis was also observed in sodium citrate-anticoagulated samples (data not shown). The EDTA-anticoagulated samples showed a progressive and marked hemolysis that was apparent from the time of sampling at room temperature and at 37°C. Both the citrated and heparinized samples also showed hemolysis at 24 hours, which exceeded the degree of hemolysis that was seen in the serum samples. No spontaneous agglutination was observed in the plasma samples at 4, 22 to 24, or 37°C.

On Day 18, the patient's Hb level dropped from 80 to 66 g per L coincident with the administration of three additional doses of cefazolin in error (Fig. 1). A search for anti-cefazolin and anti-rifampin using standard methods was negative.[1,2] The use of a polyspecific anti-human globulin reagent showed no reactivity when the patient's plasma (Days 12, 14, and 18) or eluate (Day 12) were mixed with K– RBCs coated with cefazolin after incubation for 1 hour at 37°C by both the tube and column agglutination methods. No hemolysis, agglutination, or surface IgG or C3b/d were detected using a polyspecific anti-human globulin reagent when either cefazolin or rifampin were added to aliquots containing the patient's serum, normal serum, and group O enzyme-treated or untreated RBCs. The testing for cefazolin-dependent antibodies was repeated in the American Red Cross Reference Laboratory (Los Angeles, CA) and confirmed our negative results (G. Garratty, written communication, May 2000).

After Day 20, the patient's hematologic indices began to normalize and she required no further transfusions. The patient was seen for further follow up at Days 35, 75, and 125, during which time she continued to be treated with vancomycin. The patient's complete blood count, bilirubin, and LDH were all normal when tested 75 days after the incompatible transfusion. The EDTA-dependent in vitro hemolysis was still present at Day 75, but had resolved by Day 125. The DAT was still positive with IgG when last tested at Day 125.

DISCUSSION

The mechanism(s) underlying the EDTA- citrate- and heparin-induced hemolysis are unclear. The following mechanisms may be implicated:

1. Continuing complement activation in vitro resulting in RBC hemolysis. This seems unlikely because EDTA chelates divalent cations of calcium and magnesium making them unavailable to generate the C1 and C42 (C3 convertase) complexes. A case of C3-independent bystander lysis has been reported in the literature,[3] but this is an unlikely explanation because the brisk in vivo hemolysis began to resolve after days, whereas the in vitro lysis continued for weeks.
2. A direct effect of calcium chelation on RBC membrane. Addition of calcium chelators may have direct physiochemical effects on an already weakened RBC membrane inducing further in vitro hemolysis. Calcium ions are an integral part of the RBC membrane structure and confer compactness to phospholipid membranes by increasing the packing of lipids. When calcium is chelated from RBC membranes, they become unstable and undergo a reversible shape change.[4] However, depletion of membrane-bound calcium cannot explain the hemolysis that was observed in the heparinized plasma samples.
3. RBC hemolysis by EDTA-dependent hemagglutinins. Hemagglutinins have been reported whose activity is dependent on the presence of EDTA[5] or carboxylic groups[6] or on the absence of ionized calcium.[7] Hemolysis is rare and has been reported only once.[6] In the present case, it is unlikely that hemagglutinins are implicated because no spontaneous agglutination was observed, and heparinized samples hemolysed in the absence of EDTA and carboxylic groups.

Our serologic observations are consistent with those reported by Salama and Mueller-Eckhardt[8] and Ness et al.[9] rather than with the traditional view of DHTR.[10] In this case, a positive DAT was observed for up to 125 days, suggesting the development of autoantibodies after transfusion and ongoing complement activation in vivo since C3b and C3d are unstable.[11]

The prevalence of this phenomenon is not known at the present time. The two large series of Salama and Mueller-Eckhardt[8] and Ness et al.[9] do not describe this phenomenon despite using EDTA-anticoagulated blood samples for the performance of the DAT in a total of 60 patients. We suggest that patients with an ongoing, severe DHTR should be investigated using both plasma and serum samples to detect in vivo hemolysis and to allow for the correct assessment of the rate of RBC hemolysis. Future investigations of this phenomenon could include heat inactivation or removal of complement to determine its role in the in vitro hemolysis.

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