Acute hemolytic transfusion reaction caused by anti-Yt^a

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Many patients with anti-Yt^a receive multiple transfusions of Yt(a+) red blood cells (RBCs) with no ill effects. However, anti-Yt^a has been implicated in hemolytic transfusion reactions. Antibody identification typically determines specificity of antibodies and their clinical significance to justify blood requirements for antigen-negative blood when clinically significant antibodies are involved. Occasionally, specificity of antibody is of variable significance. Variability in clinical significance is a characteristic of anti-Yt^a that may affect the clinical management of such patients. This case reports the outcome of an incompatible transfusion in an 83-year-old female patient with anti-Yt^a, -D, -C, -Le^ab, and -HI who was admitted to the hospital for a severe urinary tract hemorrhage and fever. The patient was transfused with 1 crossmatch-incompatible group A, Yt(a+), D–, C–, E–, S– RBC unit in an emergency medical event. During that time, the patient exhibited chills, shivering, and tachycardia. Decreases in hemoglobin and hematocrit were noted. Laboratory parameters for hemolysis, such as total bilirubin, direct bilirubin, and lactate dehydrogenase, were increased. Based on clinical and laboratory evaluation, it was concluded that the patient had an acute hemolytic transfusion reaction caused by anti-Yt^a. The patient was successfully treated with antipyretics, antihistamines, and corticosteroids. Urinary tract hemorrhaging was stopped. Anemia was additionally improved with parenteral iron supplementation, and further transfusion was not required. *Immunohematology* 2021;37:13–17.

Key Words: incompatible transfusion, acute hemolytic transfusion reaction, high-prevalence antigen, anti-Yt^a

The Yt^a antigen is part of the Cartwright blood group system (YT). The YT system is the 11th human blood group system recognized by the International Society of Blood Transfusion (ISBT 011), which now includes five antigens: one pair of antithetical antigens, Yt^a and Yt^b, and three additional high-prevalence antigens (HPAs): YTEG, YTLI, and YTOT.[1] YT antigens are found on the glycosylphosphatidylinositol-linked red blood cell (RBC) glycoprotein acetylcholinesterase (AChE), which plays an essential part in neurotransmission.[2] The importance of AChE is further evidenced by the lack of any known inherited Yt(a–b–) phenotype. YT antigens are encoded by *ACHE* on chromosome 7q22.

Yt^a is an HPA found in all populations.[3] It was first described in 1956 by Eaton et al.[4] Later studies showed that approximately 1 in 500 individuals of European descent are Yt(a–).[5,6] Individuals of Jewish descent and Middle Eastern ethnicities tend to have lower prevalence of Yt(a+).[7] The phenotype is less common in African Americans, with approximately 1 in 2000 Yt(a–) individuals.[8] As a result, blood lacking Yt^a is very rare and should be preserved for its most appropriate use. Anti-Yt^a is categorized into a group of antibodies with variable clinical significance and unknown hemolytic potential. Therefore, it is of outmost importance to determine those patients who require rare Yt(a–) blood to avoid hemolytic transfusion reactions (HTRs). For this purpose, *in vitro* methods, such as RBC survival studies or cellular assays, the monocyte monolayer assay (MMA), and the chemiluminescence test (CLT), have been recommended.[9–11]

Several case reports describing anti-Yt^a in patients have demonstrated rapid *in vivo* destruction of Yt(a+) RBCs.[12–14] However, Dobbs et al.[15] studied three patients with anti-Yt^a: two pregnant women without the evidence of hemolytic disease of the fetus and newborn (HDFN) and one patient with pure RBC aplasia and no HTR after transfusion of Yt(a+) RBCs. In this report, we describe a patient with an acute hemolytic transfusion reaction (AHTR) after the transfusion of 1 crossmatch-incompatible Yt(a+) RBC unit.

Case Report

In 2016, an 83-year-old white female patient of Croatian ancestry was admitted to the hospital with hematemesis from a duodenal ulcer. The patient's hospital admission was attributed to poorly controlled anticoagulant therapy for the management of deep venous thrombosis, which previously led to cerebral infarction. Admission hemoglobin (Hb) was 6.6 g/dL and medication included only warfarin. According to the medical records history, the patient had a mixture of anti-D, anti-C, and a suspected additional antibody at an outside hospital in 2005, but antibody investigation was not completed. The patient had two pregnancies and no transfusions.

Initial evaluation in year 2016 confirmed anti-D and anti-C in the patient's plasma. An antibody of unknown

specificity that was suspected in year 2005 in this patient was not detected. Initial panels for the antibody investigation included ID-DiaPanel and ID-Panel-P (Bio-Rad, Cressier, Switzerland), Panel C (Ortho Clinical Diagnostics, Raritan, NJ), and Identisera Diana, Identisera Diana P, Identisera Diana Extend, and Identisera Diana Extend P (Diagnostics Grifols, Barcelona, Spain). The patient's blood group and RBC phenotype were reported as group A, C−D−E−; K+k+; Jk(a+b+); Fy(a+b+); M+N+S−s+; and P1+ by the conventional serology tube method.

The patient received 3 units of fresh frozen plasma (FFP), immediately followed by 1 unit of crossmatch-compatible group A, C−, D−, E−, S− RBCs. The following day, 2 units of crossmatch-compatible group A, C−, D−, E−, S− RBCs were transfused for an Hb of 6.9 g/dL. Although antibody investigation confirmed only anti-D and -C to be present in the patient's plasma, RBCs units transfused were D−, C−, E−, and S−. RBC units negative for E and S were given because of the report from 2005 where another antibody specificity in addition to anti-D and -C was suspected in the patient's plasma. Assuming this antibody could have been anti-S, and to avoid alloimmunization to E, RBC units transfused were also S− and E−.

Another RBC unit was requested 6 days later for an Hb of 9.3 g/dL. The crossmatch was weakly positive. Additional studies revealed the presence of anti-D and -C and an apparent alloantibody to the HPA. Findings of the serologic investigation were communicated to the clinic. Laboratory parameters were negative for hemolysis (total bilirubin [TB] 20 μmol/L, normal values 3–20 μmol/L; lactate dehydrogenase [LDH] 217 U/L, normal values <241 U/L). Therefore, HTR was not suspected. Because the clinical condition of the patient was stable, the attending physician cancelled the transfusion request that was sent to further improve anemia. The next day, new samples were requested, but the patient had already been discharged from the hospital.

Obtaining new blood samples was coordinated by the patient's caregivers. The patient was living in a nursing home, at which the nurses collected and arranged the transport of samples to the hospital. From the hematologic status at the nursing facility, the patient's Hb was 12.7 g/dL.

A few weeks later, samples that had been collected while the individual was in outpatient care were received for antibody identification and sent to the International Reference Laboratory (IRL). In the plasma of the patient, the following complex mixture of antibodies was revealed: anti-Yt[a], -D, -C, -Le[ab], and -HI. A variety of methods, including alloadsorption-elution studies and inhibition tests using soluble recombinant Yt[a] proteins, were used. At the IRL, genomic DNA was isolated from the whole blood. Polymerase chain reaction amplification and Sanger sequencing of all coding exons of erythroid isoform of *ACHE* were carried out. The results showed that the patient had the *YT*B* genotype and, therefore, a Yt(a−b+)-predicted phenotype.

The patient was again hospitalized in 2019 for hematuria, caused by over-anticoagulation, and fever as a consequence of urinary tract infection. A total of 3 units of FFP were transfused, after which 2 RBC units were ordered from the emergency department for severe anemia (Hb 6.5 g/dL). Crossmatching and initial panels for antibody identification gave equally positive reactions (2+) with all RBCs tested by low-ionic-strength saline solution (LISS)–indirect antiglobulin test (IAT). Findings of the serologic testing and risks of anti-Yt[a] associated with the incompatible transfusion were communicated to the clinic. Because of the rarity and complexity in obtaining Yt(a−) RBC units, the suggestion was made to cancel the RBC transfusion. Based on severe urinary tract hemorrhage, anemia, and the severely disturbed general condition of the patient, the attending physician decided to transfuse the patient with 1 crossmatch-incompatible RBC unit. In addition to the urinary tract hemorrhage and anemia, the patient had pneumonia, a urinary tract infection, and was febrile, dehydrated, somnolent, hypo-oxygenated, and hypotensive.

Thirty minutes after premedication (which consisted of 80 mg methylprednisolone and 1 ampoule of chloropyramine), the patient was transfused with 1 crossmatch-incompatible group A, C−, D−, E−, S− RBC unit. There was no possibility to do additional testing, or to search for a compatible or the least incompatible unit at the time because the blood transfusion was needed during night hours over the weekend. The transfusion was stopped after the patient had received 130 mL RBCs because she exhibited chills, shivering, and tachycardia, which were not present before transfusion. A sample was drawn immediately after the transfusion was stopped. Under the suspicion of an AHTR, the pre- and post-reaction samples were evaluated.

In the pre-transfusion antibody study, the crossmatch was incompatible with the selected group A, C−, D−, E−, S− RBC unit. A pre-transfusion direct antiglobulin test (DAT), using anti-IgG and anti-C3d, was negative. ID-Card LISS/Coombs cards with polyspecific antihuman globulin (rabbit anti-IgG and monoclonal anti-C3d, cell line C139-9) and DC-Screening II cards with monospecific anti-IgG and -C3d (Bio-Rad) were

used for the DAT. In evaluation studies, the patient's plasma was adsorbed with three randomly selected C+, D+, E–, S– RBCs to remove anti-D, -C, and -Yt^a. The presence of new alloantibodies in the patient's plasma was excluded by LISS-IAT with untreated and papain-treated RBCs. The donor's RBCs were retested with the patient's adsorbed plasma to determine whether any additional antibodies existed apart from the anti-Yt^a. Negative results confirmed that no additional antibodies were present in the patient's plasma, including antibodies to low-prevalence antigens. At the IRL, the patient's plasma was tested with two examples of D–, C–, Le(a–b–), Yt(a–) untreated RBCs by LISS-IAT that were compatible, confirming anti-Yt^a in the patient's plasma and excluding additional antibodies, such as antibodies to HPAs. Patient's plasma was also tested with Yt(a–), Le(a+) RBCs to detect anti-Le^a; anti-Le^a was not detected by the LISS-IAT.

In the post-transfusion antibody study, the crossmatch was incompatible with the selected group A, C–, D–, E–, S– RBC unit. Post-transfusion DAT was positive: anti-IgG 1+; anti-C3d negative. An acid eluate was performed using DiaCidel (Bio-Rad). Anti-Yt^a was found in the eluate, reacting moderate strength by LISS-IAT with untreated RBCs and marginally weaker with papain-treated RBCs. Post-transfusion eluate was tested additionally with Yt(a–), Le(a+) RBCs; anti-Le^a was excluded. The eluate was then adsorbed to remove anti-Yt^a. No other additional antibodies were detected in the eluate. Also, no new antibodies were detected in the serum of the post-transfusion sample after alloadsorption with three randomly selected C+, D+, E–, S– RBCs.

The results of the transfusion reaction evaluation are shown in Table 1. The following laboratory findings suggested an AHTR: decreased levels of Hb and hematocrit (Hct), and increased levels of TB, direct bilirubin, and LDH (Table 2). The patient was successfully treated with antipyretics, antihistamines, and corticosteroids. Over-anticoagulation was resolved, and urinary tract hemorrhaging ceased. Parenteral

Table 1. Transfusion reaction evaluation

Laboratory evaluation	Pre-transfusion	Post-transfusion
ABO and D	Group A, D–	Group A, D–
Visual check of the plasma	Negative for hemolysis	Negative for hemolysis
Antibody detection test	Positive	Positive
Crossmatch with the transfused RBC unit	2+	2+
DAT (anti-IgG)	Negative	1+

RBC = red blood cell; DAT = direct antiglobulin test; Ig = immunoglobulin.

Table 2. Selected laboratory values in the patient

	Patient's results			
Laboratory test	Before transfusion	After transfusion	After 11 days	Normal range
Hemoglobin (g/dL)	6.5	5.7	9.5	11.9–15.7
Hematocrit (%)	20.9	18.8	28.8	35.6–47.0
Reticulocytes (10^9/L)	NT	113	139	22–97
Total bilirubin (μmol/L)	20	46	16	3–20
Direct bilirubin (μmol/L)	NT	14	NT	<5
Lactate dehydrogenase (U/L)	200	353	270	<241
Haptoglobin (g/L)	NT	1.26	NT	0.30–2.20
Urine analysis				
Proteins	3+	Negative	NT	Negative
Urobilinogen	1+	Negative	NT	Negative
Erythrocytes	3+	3+	NT	Negative

NT = not tested.

iron supplementation was implemented, the patient's anemia was improved, and further transfusion was not required.

Discussion

An AHTR may occur when RBCs are transfused to a patient with a preexisting antibody that destroys the transfused incompatible RBCs. AHTR is characterized by a rapid destruction of RBCs immediately after transfusion.

Our patient was transfused during the night over the weekend in an emergency medical event with 1 crossmatch-incompatible group A, Yt(a+), C–, D–, E–, S– RBC unit, because there was no time to obtain Yt(a–) blood or to search for the least incompatible RBC unit. All findings were consistent with AHTR. The patient suffered chills, shivering, and tachycardia. Laboratory parameters for hemolysis were positive including decreases in Hb and Hct, as well as an increase in TB, direct bilirubin, and LDH. DAT was positive. At the time of admission, the patient had hematuria, and, as a result, hemoglobinuria could not be analyzed separately. In the post-transfusion eluate, anti-Yt^a was confirmed. No additional antibodies were detected. It is known that anti-HI can rarely cause delayed hemolysis mostly with group O RBCs, which is highly unlikely in this case, since the patient was transfused with group A RBCs. Lewis antibodies, particularly anti-Le^a, seldom cause HTR if they are reactive at 37°C by the IAT.[2] The risk of HTR is lower if Le(a+) RBCs of group A are transfused

since group A RBCs express fewer Lewis antigens than group O RBCs.[16] In the case presented here, anti-Le[a] was not reactive at 37°C by the IAT and therefore was considered non-significant. Group A RBCs were also transfused to the patient. Involvement of anti-Le[a] in the HTR was finally excluded by testing the post-transfusion eluate. From the transfusion reaction evaluation, we concluded that the transfusion reaction was caused by anti-Yt[a]. All other possible causes for the AHTR, such as thermal destruction of RBCs and concomitant administration of drugs, were excluded, since the patient was not transfused through blood warmers, and she did not receive any drugs during the transfusion. Our patient did not receive further RBC units during hospitalization because her anemia improved when over-anticoagulation was resolved and urinary tract hemorrhaging ceased. Anemia was further improved with parenteral iron supplementation.

This case describes an instance of an AHTR caused by anti-Yt[a]. Previous reports have described rapid *in vivo* destruction of Yt(a+) RBCs in patients with anti-Yt[a] using the [51]chromium (^{51}Cr) labeling survival study.[12–14] A case study by Bettigole et al.[12] documented the injection of a small volume of ^{51}Cr-labeled Yt(a+) RBCs into a prostatic carcinoma patient with anti-Yt[a]. The half-life of the transfused RBCs at 12 minutes was estimated to be 3 days. Anti-Yt[a] was concluded to have the potential to cause an HTR.[12] In the report by Göbel et al.,[13] shortened survival of injected ^{51}Cr-labeled Yt(a+) RBCs was induced by anti-Yt[a] in a mother immunized during her second pregnancy, but the anti-Yt[a] did not cause HDFN. The third case reports anti-Yt[a] with rapid destruction of injected [51]Cr Yt(a+) RBCs. It was concluded that transfusion of Yt(a+) blood would result in an HTR.[14] Although previous reports of anti-Yt[a] suggested it to be a clinically significant antibody concerning transfusion, some patients with anti-Yt[a] received Yt(a+) blood without apparent reaction.[4,15] In the report by Eaton et al.,[4] anti-Yt[a] was detected in the patient 4 days after a second series of transfusions. Although there was a weakly positive DAT 60 days after transfusion, it was concluded that Yt(a+) RBCs were still present and that anti-Yt[a] did not reduce the survival of transfused RBCs.[4] Dobbs et al.[15] studied one patient with pure RBC aplasia and no HTR after transfusion of Yt(a+) RBCs. This finding may be explained by the immunoglobin G (IgG) subclass composition of the anti-Yt[a] cases, as an *in vitro* method used to assess the clinical significance of RBC antibodies. Reported examples of anti-Yt[a] showed that it is almost always of the IgG class.[17,18] According to these studies, the presence of IgG1 as well as IgG4 are characteristic of anti-Yt[a].[17,18] IgG subclass composition, especially the absence of

IgG3, may explain the often-benign effects of anti-Yt[a]. It is well known that IgG3 is thought to be the driving force behind AHTR. Therefore, severe reactions should be expected where IgG3 is implicated. IgG subclassing of our patient's anti-Yt[a] was not carried out because it was not available.

AuBuchon et al.[19] demonstrated a change in clinical significance of anti-Yt[a]. In that study, the ability of the patient's anti-Yt[a] to shorten the lifespan of ^{51}Cr-labeled Yt(a+) RBCs increased almost threefold, 3 months after transfusion with 4 units of Yt(a+) RBCs. The change occurred several years after the initial appearance of the anti-Yt[a], so this change would not be related to a progression in antibody class or subclass evolution as part of a primary antibody response. The change in clinical significance was due rather to the appearance of an IgG1 component of the anti-Yt[a], as a response to the further transfusion of RBCs to which the patient had been previously sensitized. Mohandas et al.[20] suggested considerable variability in the tolerance of Yt(a+) transfusions of patients with anti-Yt[a]. Based on their experience, transfusion of Yt(a+) RBCs into patients with anti-Yt[a] is likely to be safe, more often than not. Nevertheless, patients with anti-Yt[a] should not be transfused routinely with Yt(a+) RBCs. Antibodies to Yt[a] have not caused HDFN, despite several cases of women with anti-Yt[a] identified after having Yt(a+) children. This finding may be partly explained due to weak expression of Yt[a] on fetal RBCs.[21,22]

Because anti-Yt[a] has shown variable significance in the literature, cellular assays can be performed to ascertain the clinical significance of anti-Yt[a]. The MMA measures the adherence and phagocytosis of sensitized RBCs by human monocytes, which is determined microscopically. The CLT measures the metabolic bioproducts associated with the phagocytosis of sensitized RBCs using luminol to produce light. MMA is found to be a reliable predictor of the clinical significance of anti-Yt[a].[10,23] Eckrich et al.[23] conducted a retrospective review of MMA results on sera from 79 patients with anti-Yt[a]. They found that MMA is a reliable predictor of the clinical significance of anti-Yt[a], when samples for analysis were collected at least 6 weeks after initial detection of the antibody.[23] Results using the CLT suggested that it might be used in a similar manner to the MMA, to predict an outcome of transfusing incompatible blood.[11] According to Hadley et al.,[11] results from MMA and CLT are in good agreement, although some sera were strongly reactive in the MMA and weakly reactive in the CLT. Sera that promote RBC adherence more than phagocytosis may be more reactive in the MMA.[11] MMA is available in several laboratories to predict the survival of

transfused incompatible RBCs, but it is currently not available in Croatia.

In conclusion, for transfusion purposes, cellular assays, such as MMA or CLT, should be performed when time permits to determine the clinical significance of anti-Yt[a]. Based on these results, rare Yt(a−) blood units should be obtained only for the patients with clinically significant anti-Yt[a]. Because IgG subclass may be related to clinical significance of anti-Yt[a], IgG subclassing is recommended when possible.

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