Anti-Kp^a–induced severe delayed hemolytic transfusion reaction

R. Koshy, B. Patel, and J.S. Harrison

Kp^a is a low-frequency antigen occurring in less than 2 percent of the Caucasian population. Mild to moderate delayed hemolytic transfusion reactions (DHTR) and hemolytic disease of the fetus and newborn attributable to anti-Kp^a have been reported. Severe overt DHTR has not been reported with anti-Kpa. A case of a severe DHTR attributed to anti-Kp^a after multiple RBC transfusions is being reported. A 52-year-old Caucasian woman received multiple units of RBCs for a lower gastrointestinal bleed. She was referred to our institution for hepatic and renal failure, which was supported by laboratory findings of peak LDH, bilirubin, BUN, and creatinine elevations. Hemoglobin had dropped on Day 10 after transfusion. The DAT and antibody screen (ABS) were negative. Initial workup and subsequent ABS were negative. Anti-Kp^a was identified when an additional RBC panel was tested. One of the RBC units transfused was incompatible by antihuman globulin (AHG) crossmatch with the patient's plasma and typed positive for Kp^a. DHTR was confirmed after extensive workup. The patient responded to supportive therapy and experienced an uneventful recovery. DHTR may not be considered when DAT and ABS are negative. However, correlation of recent transfusion with signs and symptoms should alert the clinician to entertain and investigate a DHTR that should include the AHG crossmatch of all implicated RBC units. The severity of the reaction also raises concerns as to when and what antigen specificity should be considered for inclusion in the antibody screening cells.

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Kp^a (KEL 3, Penney) is one of 31 antigens in the Kell (ISBT symbol, KEL) blood group system. The Kell antigens, encoded by *KEL*, located on chromosome 7q33, appear to be erythroid specific and are found in the fetal liver and in bone marrow cells.[1] Kp^a is a low-incidence antigen found in less than 2 percent of Caucasians.[1,2] The antigen is resistant to the effects of enzyme treatment but is sensitive to treatment with dithiothreitol and acid. Mild to moderate delayed hemolytic transfusion reaction (DHTR) and mild to moderate hemolytic disease of the fetus or newborn (HDFN) have been reported as well as a case of hydrops fetalis attributed to anti-Kp^a.[3] The antibody is an IgG.[4–6] Suppression of erythropoiesis by anti-Kp^a has been reported as the cause of decreased hemoglobin in HDFN.[7] Reports of an overt DHTR attributable to several antibodies missed in the antibody screen and immediate-spin crossmatch mention anti-Kp^a as one of the antibodies.[8] However, there have been no reported cases of severe overt DHTR as a result of anti-Kp^a as per MEDLINE search.

Case Report

A 52-year-old Caucasian woman presented to the emergency room complaining of being light-headed and having rectal bleeding, nausea, weakness, and dizziness. Rectal examination confirmed the rectal fissures and bleeding. There were no other remarkable findings noted on physical examination. ECG showed sinus tachycardia at 106 beats/min. She was admitted for further workup and for RBC transfusions.

Past history was negative for prior transfusions. Patient has an adult son who was listed as a contact person, and no other pregnancy history was available. She had left renal angioplasty several years ago for left renal artery stenosis. She had a history of rectal fissures. She is a smoker (2 packs per day) and drinks occasionally. She has a history of allergy to amitriptyline.

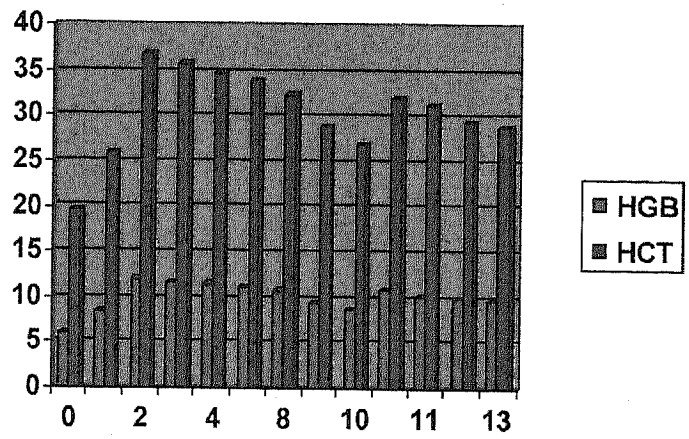

Fig. 1 Hemoglobin (Hb; g/dL) and hematocrit (Hct; %) values for day 0 to day 13 are shown.

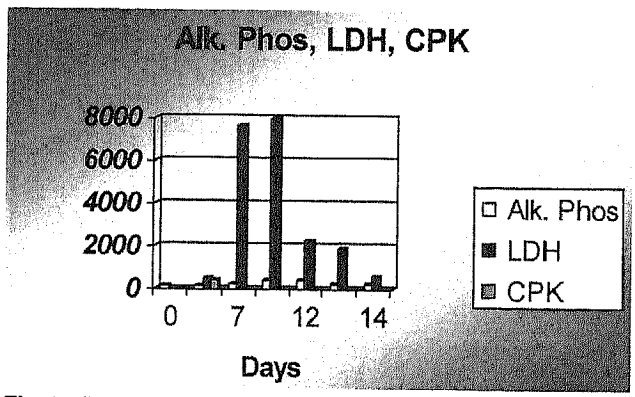

Fig. 2 Alkaline phosphatase (Alk Phos), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) values for day 0 to day 14 are shown.

Table 1. Laboratory data from the referring hospital

Tests	Normal range	Patient results					
		Admission	Day 2	Day 7	Day 10	Day 11	Day 12
Hemoglobin	4.5–11 g/dL	6.1	12	—	8.7	10.2	9.9
Hematocrit	36%–48%	19.6	—	—	26.9	31.2	29.3
WBC	4.5–11.0 x 10^9/dL	9.9	—	—	—	—	—
Platelets	120,000–450,000/μL	628,000	—	—	—	—	—
Reticulocytes	0.9%–1.9%	—	—	—	—	4.1	—
PT	11.5–14.7 s	13.8	—	—	13.5	15.2	18.1
Glucose	65–100 mg/dL	125	—	—	—		
Alkaline phosphatase	30–133 U/L	138	142	203	211	422	393
BUN	7–21 mg/dL	18	—	18	36	46	51
Creatinine	0.5–1.4 mg/dL	0.8	—	1.0	4.2	5.6	6.2
AST	5–39 U/L	31	—	—	161	529	303
ALT	7–56 U/L	19	—	—	113	168	120
LDH	333–699 U/L	—	521	3983 7637	—	7905	2215
CPK	35–230 U/L	87	425	—	—	—	—
Total bilirubin	0.2–1.3 mg/dL	0.3	—	—	1.8	23.5	18.3
Direct bilirubin	0.0–0.4 mg/dL	—	—	—	—	24.7	16.1
ABO, D	—	O D positive	—	—	O D positive	O D positive	—
Antibody screen	—	Negative	—	—	Negative	Negative	—
DAT	—	—	—	—	—	Negative	—
HBsAg, HBc antibody, HBs antibody, and hepatitis C antibody—all nonreactive							
Urinalysis		—	—	—	—		—
Bilirubin	Negative					Moderate	
Urobilinogen	<1 mg/dL					4 mg/dL	
Free hemoglobin	Negative					Large	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CPK = creatine phosphokinase; HBc antibody = hepatitis B core antibody; HBs antibody = hepatitis B surface antibody; HBsAg = hepatitis B surface antigen; PT = prothrombin time; WBC = white blood cell count.

Admission Hb was 6.1 g/dL. She was transfused with 5 units of type-specific, leukoreduced or washed (as per hospital policy), immediate-spin, crossmatch-compatible RBC during the course of 24 hours of admission, which raised her Hb to 12 g/dL. She remained hospitalized for further evaluation for gastrointestinal bleed. On the 10th posttransfusion day, her Hb dropped to 8.7 g/dL and her Hct was 26.9%. Her reticulocyte count was 4.1% (reference range, 0.9 to 1.9%) on Day 11. There was no evidence of continued rectal bleed. There was a gradual increase of several chemistry results, which peaked on the 10th and 11th posttransfusion days (Table 1; Figs. 1–4). The patient appeared severely jaundiced with signs of acute liver and renal failure, which was supported by her laboratory values. Investigation for a suspected DHTR revealed a negative DAT and a negative antibody screen. Investigation did not proceed to antihuman globulin (AHG) crossmatch for all RBC units transfused. Urinalysis on Day 11 showed evidence of increased free hemoglobin, bilirubin, and urobilinogen. Computed tomographic scan and magnetic resonance imaging to rule out acute cholecystitis revealed an enlarged gallbladder with thickened wall and inflammation of adjacent areas, reported as suspicious for cholecystitis. Liver, spleen, and pancreas were within normal limits. There was also evidence of atrophic left kidney and hypertrophic right kidney with severe stenosis of a segment of the right renal artery close to its origin. The patient received 3 additional units of RBCs between Days 10 and 11 for the drop in hemoglobin.

On the 13th day of her hospital course, she was admitted to the referral hospital, our institution, for investigation of acute liver and renal failure. DHTR was suspected, and a workup was ordered by the hematologist. Pigment nephropathy attributable to intravascular hemolysis was considered as the cause for the acute renal failure. The cause of the severe hepatotoxicity could not be ascertained.

Materials and Methods

At the referral hospital, ABO and D typings were performed using the Ortho A/B/D Monoclonal and Reverse Grouping Card (Ortho-Clinical Diagnostics, Inc., Raritan, NJ). Affirmagen reagent RBCs (pooled cells), 0.8%, were used for reverse typing, and antibody screen was done by IgG gel card, 0.8% Surgiscreen (Johnson & Johnson, New Brunswick, NJ). Antibody panel was performed using 0.8% Resolve Panel A (Ortho-Clinical Diagnostics, Inc.) and Panocell 16, by Immucor, Inc. (Norcross, GA). The DAT was performed using anti-IgG-C3d with check cells, anti-IgG and check cells, and anti-C3b/C3d with complement check cells (Immucor, Inc.). Reagents used for phenotyping were from Immucor, Inc. Elution was performed with Gamma ELU-KIT II (Gamma Biologicals, Inc., Houston, TX) following initial saline wash (Fisher Diagnostics, Middleton, VA) of patient cells.

ABO and D typings and a three-cell antibody screen were performed by gel technique and read through the MTS reader SA (Ortho-Clinical Diagnostics, Inc.). DAT was performed with polyclonal AHG, anti-IgG, and anti-C3d by tube method and eluate panel by gel method using Ortho and Immucor panel cells.

Results

The DHTR investigation on the day of admission at the referral hospital revealed a negative antibody screen with the three-cell antibody screen. The DAT was negative (including a 15-minute incubation) with polyspecific, IgG, and C3b/C3d AHG. At the referral hospital, tests with a 16-cell reagent RBC panel (Immucor, Inc.) revealed the presence of anti-Kp^a. All other clinically significant antibodies were ruled out. Eluate prepared from DAT-negative RBCs was negative against Kp(a+) RBC on Immucor Panocell 16. The patient typed negative for Kp^a. AHG crossmatch was done from samples obtained from donor segments of the transfused RBC units received from the blood supplier. One of the five RBC units transfused on Day 1 of admission was incompatible with the patient's plasma and typed positive for Kp^a. Haptoglobin was 1 mg/dL (reference range, 34–200 mg/dL). A diagnosis of severe DHTR with intravascular hemolysis attributable to anti-Kp^a was confirmed. As the patient's renal and liver functions improved, she did not undergo dialysis or consideration for further liver or renal evaluation.

The patient was monitored and maintained on supportive therapy. Her hospital course was uneventful. All laboratory results returned to normal values (Figs. 1–4). She was discharged 10 days after her admission to the referral hospital.

Table 2 presents the patient's laboratory data on admission to the referral hospital on Day 13.

Table 2. Laboratory data on admission at the referral hospital, Day 13

Test	Result
Haptoglobin (reference range 34–200 mg/dL)	1 mg/dL
ABO, D (by Ortho gel)	O, D positive
Antibody screen (0.8% Surgiscreen)	Negative
Panel cells, A	Negative
Panocell 16	Positive (anti-Kp^a identified)
Kp^a typing of patient's RBCs	Negative
DAT—using polyclonal, IgG, and C3b/C3d AHG	Negative
Panel with eluate	Negative
Crossmatch with 1 of 5 units transfused at referring hospital	Incompatible, Kp(a+)
Kp^a typing of incompatible unit	Kp(a+): 1+ by tube and 2+ by gel techniques

Discussion

Kp^a was first described by Allen and Lewis in 1957.[2] The Kp^a antigen is a member of the Kell blood group system and is carried on the Kell glycoprotein. The prototypical gene for the Kell protein family was cloned and characterized in the early 1990s.[9] As discussed by Lee and colleagues,[9] the antigens of the Kell blood group system result from single nucleotide changes in the Kell protein. Most Kell antigens

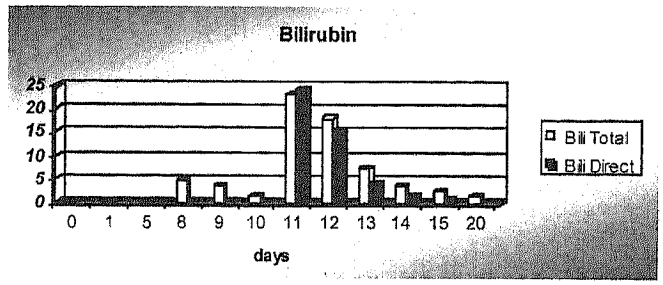

Fig. 3 Total and direct bilirubin values for day 0 to day 20 are shown.

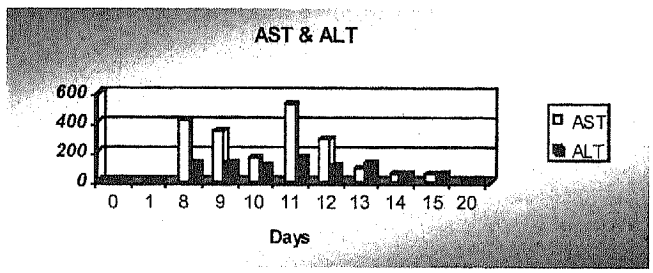

Fig. 4 Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values for day 0 to day 20 are shown.

reside on the *C*-terminal domain of Kell in the structural sequence *N*-terminal to the zinc-binding catalytic motif, which is the major site for endothelin-3–converting enzyme activity.

Kell antigens are important in transfusion medicine owing to their strong immunogenicity, and the corresponding antibodies are clinically significant.[9] Anti-Kp^a has been implicated in mild to moderate HDFN and DHTR. Severe DHTR as presented in this case has not been reported. Clinical signs and the laboratory values presenting on Day 7 with peak laboratory values at Days 10 and 11 after transfusion of a unit of RBCs positive for Kp^a support a diagnosis in this case of a severe DHTR with intravascular hemolysis attributable to anti-Kp^a. The cause of the primary Kp^a exposure could not be determined from the patient's history. AHG crossmatch of all implicated RBC units despite the negative DAT or antibody screen would have positively identified the incompatible Kp(a+) RBC as the implicated unit for the DHTR.

The patient under discussion experienced unusually severe hepatotoxicity, and the reasons for this remain unclear: there was no prior history of liver disease. Severe hemolysis with resultant high levels of free heme can cause undesirable toxicity, leading to organ, tissue, and cellular injury. The mechanism of action is through free heme that catalyzes the oxidation, covalent crosslinking, and aggregate formation of protein and its degradation to small

peptides. It also catalyzes the formation of cytotoxic lipid peroxide via lipid peroxidation and damages DNA through oxidative stress. Heme, being a lipophilic molecule, intercalates in the membrane and impairs lipid bilayers and organelles, such as mitochondria and nuclei, and destabilizes the cytoskeleton. It also causes endothelial cell injury, leading to vascular inflammation, and stimulates the expression of intracellular adhesion molecules. As a proinflammatory molecule, heme induces inflammation that results in toxic effects on the kidney, liver, central nervous system, and cardiac tissue.[10] The severe heme toxicity may also be attributable to the markedly compromised renal condition owing to the severe stenosis of the right renal artery.

The severity of the DHTR in this case may warrant consideration for inclusion of clinically significant low-frequency antigens that may be missed by current screening cells for detection of clinically significant RBC antibodies. However, the risk of overt DHTRs to low-incidence antigens is estimated at 1 per 650,000 crossmatches.[5] The calculation was based on report of probability of acute overt hemolytic transfusion reaction at 1 per 260,000 with immediate-spin crossmatches of 1.3 million negative antibody screens.[8] As the risk of overt DHTR is extremely small, the cost benefit should be considered for inclusion of low-frequency antigens such as Wr^a, C^w, and Kp^a in the antibody screening cells.[5] We concur with the previous observation. AHG phase crossmatch must be included in the investigation of any suspected DHTR irrespective of a negative DAT or negative antibody screen. Appropriate and timely patient care to avert further patient harm depends on a thorough investigation.

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Ranie Koshy, MD (corresponding author), Director, Blood Bank and Transfusion Services, and Bhishma Patel, SBB, Blood Bank and Transfusion Safety Officer, Department of Pathology and Laboratory Medicine, NJMS/UMDNJ, C101, University Hospital, Newark, NJ 07103; and Jonathan S. Harrison, MD, Department of Medicine, Hematology, Robert Wood Johnson Medical School—Pisc/New Brunswick, New Brunswick, NJ.