

K antigens on neonatal red blood cells blocked by anti-K with titer of 32

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The blocking of red blood cell (RBC) antigens occurs when potent maternal antibodies bind to antigens on fetal or neonatal RBCs, causing them to be negative when typed with human IgM antisera. This phenomenon is rare; when it does occur, the antibody is usually of a high titer. This reported finding is typically due to anti-D, with rare reports describing false-negative K phenotyping due to blocking by maternal anti-K. We report a case of a potent anti-K with a titer of 32 that blocked K antigens on neonatal RBCs, causing them to phenotype as K-. The neonate also had clinically significant anemia (i.e., hemolytic disease of the newborn) due to the anti-K. *Immunohematology* 2020;36:54–57.

Key Words: HDFN, blocked antigen, maternal anti-K, titer

Hemolytic disease of the fetus and newborn (HDFN) is one of the serious causes of perinatal morbidity and mortality. It occurs because of a difference in red blood cell (RBC) antigens between the mother and fetus. The mother is alloimmunized during pregnancy or after blood transfusion. The mother's antibodies, when of the IgG class, enter the placental circulation, bind to fetal antigens on the RBC surface, and cause hemolysis and/or anemia as a consequence of erythropoiesis suppression.

The Kell blood group system is very polymorphic, with 35 antigens assigned to the system,¹ encoded by the *KEL* gene, with K and k being the most common antigens. K is completely expressed on fetal RBCs by the 10th gestational week. The total number of K antigen sites per RBC is quite low, but despite its lower quantity, K is very immunogenic.² Anti-K is the third most common specificity of RBC alloantibody involved in causing HDFN, with the first being naturally occurring ABO, followed by immune anti-D. About 9 percent of the white population are K+.

Anti-K causes erythropoiesis suppression and the destruction of erythroid progenitors, with consequent severe anemia and reticulocytopenia and only mild hyperbilirubinemia.³ The antibody titer does not necessarily correlate to the clinical severity of HDFN.^{2,4}

Neutralization in immunohematology is defined as a blocking of antibody sites, causing a false-negative antihuman globulin (AHG) phase of testing.⁵

First described in 1944,⁶ blocked RBC antigens were found to be due to a potent maternal anti-D that blocked D antigens on fetal RBCs, causing them to test as negative when typed with human IgM anti-D. Typing fetal RBCs with reagent antisera produced false-negative results, because all antigen sites were saturated by the antibody and unable to react with the antisera. This phenomenon is rare.⁷

Other blood group antibodies can also cause the blocking phenomenon; there are case reports of anti-K and anti-Fy^{a8} that caused the blocked antigen phenomenon.

False-negative phenotyping makes laboratory workup in a patient with HDFN more difficult. An accurate phenotype is important for fetuses and neonates who need RBC transfusion. Direct antiglobulin test (DAT) and eluate results should help identify the coating antibody. Methods described to remove IgG from RBCs are acid/EDTA elution, heat elution, and use of chloroquine diphosphate (CPD). The acid/EDTA elution method gives the best results for typing RBCs coated with warm-reactive IgG allo- or autoantibody, but it destroys K antigen expression. CPD is not always effective in eluting the antibody.^{9,10}

Molecular genotyping is not always available, and it is not as fast as the other methods.

So far, there have been four case reports that describe false-negative K phenotyping due to blocking by maternal anti-K.^{10–13}

We present a case report of a potent anti-K with a titer of 32 that caused this blocking phenomenon.

Case Report

A 29-year-old mother (G5P3A2) delivered a term male neonate by emergency caesarean section. In the 38th week of gestation, growth retardation was noticed, and labor was induced because of cardiotocography decelerations and oligohydramnios.

At delivery, the neonate weighed 2410 g and measured 46 cm long, and the Apgar score was 9 of 10. Soon after birth, the neonate required a RBC transfusion because of significant

anemia (Hb 79 g/L; normal range 136–199 g/L). The neonate's RBCs were typed as group AB, D+ and were DAT positive (3+) (Table 1).

Table 1. Changes in RBC count and bilirubin level in a neonate with HDFN caused by anti-K with a titer of 32

Age	Laboratory value				
	RBC ($\times 10^{12}/L$)	Hb (g/L)	Hematocrit (L/L)	Reticulocytes (%)	Bilirubin ($\mu\text{mol/L}$)
	Normal range				
	3.90–5.50	136–199	0.391–0.585	20–60	3–20
1 day	2.06	79	0.24	NT	NT
2 days	2.22	78	0.25	82.9	112
2 days (after transfusion of 70 mL RBCs)	4.03	128	0.39	51.4	109
3 days	4.29	136	0.41	56.4	89
6 days*	3.48	103	0.32	39.8	NT
1 month	3.27	88	0.26	7.9	NT
1 month and 4 days†	4.91	132	0.39	7.1	27
2 months	3.81	106	0.31	NT	NT
5 months	5.27	139	0.39	NT	NT

*After this complete blood count, the neonate received 50 mL RBCs and was discharged from hospital.

†After transfusion of 50 mL RBCs.

RBC = red blood cell; HDFN = hemolytic disease of the fetus and newborn; Hb = hemoglobin; NT = not tested.

The mother's blood sample was typed as group A, D+; her antibody detection test by the indirect antiglobulin test (IAT) was positive, and the antibody specificity was anti-K. From her gynecology history, we noted she previously delivered two healthy female neonates and had two missed abortions—one in the 12th and the other in the 16th week of pregnancy. She never received any blood components.

Regular screening of atypical antibodies in the antenatal period can prevent or minimize complications in alloimmunized mothers and their children. During the current pregnancy, obstetric follow-up was performed regularly, except for immunohematology testing. In Croatia, it is standard practice to perform the IAT twice in each pregnancy: at the end of the first trimester and then at the beginning of the third trimester. In this case, the mother's IAT was not checked between her first pregnancy and third delivery.

The anti-K was first identified after the delivery of her third neonate, from her fifth pregnancy. The mother's RBCs were phenotyped as K– (K–k+), the father's RBCs typed as K+ (K+k+), and the neonatal sample tested at first as K– (Table 2).

Table 2. Immunohematological workup results

Test	Neonate	Mother	Father
Blood type	Group AB, D+	Group A, D+	NT
IAT	NT	Positive	NT
Identification	NT	Anti-K	NT
Antibody titer	NT	32	NT
DAT poly AHG	3+	NT	NT
DAT anti-IgG	2+	NT	NT
DAT anti-C3d	1+	NT	NT
Eluate	Anti-K	NT	NT
Initial K phenotype	Negative (k+k+)	k+k+	K+k+
<i>KEL</i> genotype	K1K2	NT	NT
Repeated K phenotype	Positive (K+k+)	NT	NT

NT = not tested; IAT = indirect antiglobulin test; DAT = direct antiglobulin test; AHG = antihuman globulin.

DAT performed on neonatal RBCs was positive (3+), with anti-IgG (2+) and anti-C3d (1+); the eluate contained anti-K (2+). Neonatal RBCs from a peripheral venous blood sample were typed as K– with monoclonal anti-K (Diaclone MS-56, Cressier FR Switzerland). A neonatal sample was also tested by polymerase chain reaction–sequence-specific primer (PCR-SSP) with Inno-train's RBC-Ready Gene kit (Kronberg, Germany) and was confirmed to be K1K2. We had only a few milliliters of the neonate's blood sample, which we used for phenotyping, acid elution, and genotyping. Our laboratory has no practical experience in phenotyping after elution of the antibody or in using the CPD method. The molecular method was available and completed relatively quickly.

The titer of anti-K in the mother's sera in the antihuman globulin phase by the tube method with K+K+ RBCs was 32 (score 37). In room temperature and 37°C phases, the titer was zero. The sample was tested for immunoglobulin subclasses of anti-K using a DAT IgG1/IgG3 card (DiaMed, Cressier FR Switzerland), and positive results were obtained with total anti-IgG and -IgG1 at titers of 10 and 100, respectively, which is a characteristic of anti-K.

Soon after the first testing, the neonate was transfused with K– RBCs, and repeated K typing was not reliable. The neonate's anemia persisted, and he required further RBC transfusion on two more occasions. The neonate also had mild hyperbilirubinemia, ranging from 89 to 112 $\mu\text{mol/L}$ (normal range 3–20 $\mu\text{mol/L}$) which was treated with phototherapy. Repeat typing was performed 3 months after the last transfusion, when the DAT became negative, and showed that the infant was K+ (K+k+). At the age of 5 months, the infant

had a normal complete blood count and did not require any therapy.

Discussion

Anti-D is the most common specificity implicated in the blocking phenomenon. Where it does occur, the IgG antibody is usually of a titer higher than 256.^{7,14,15}

The lowest titer causing blocked K antigens reported in the literature to date is 128.¹⁰ Titration values can provide information about the relative amount of antibody present in a serum sample or the relative strength of antigen expression on RBCs.¹⁶

Case reports have shown that the clinical finding of HDFN due to anti-K does not necessarily correlate the antibody titer with the amount of blocking of the antigen sites. As previously mentioned, the number of K antigen sites per a Kk RBC is relatively low—between 2100 and 5400.¹⁷ The Kell glycoprotein antigen has a large extracellular domain on RBCs. Lee et al.¹² reported a case of blocked K caused by maternal anti-K at a titer of 256; the DAT was 5+ with cord RBCs but the neonate did not show any clinical signs of HDFN. Experiments were performed to simulate this blocking effect; the results showed that potent anti-K sera at a level of 256 or greater were capable of blocking the K antigen on K+ RBCs.¹² Hannon et al.¹⁰ reported a case with blocking phenomena due to anti-K with a titer of 128, but HDFN manifested only as mild hyperbilirubinemia, without the need for RBC transfusion.

In the case report of Manfroi and Velati¹³ in which the titer of anti-K was 256, the fetus had severe anemia and received three intrauterine RBC transfusions. The most recent case report, a pre-proof article from Moosavi et al.¹¹ discussing the blocked K phenomenon, claims that high-titer antibodies are the cause of a false-negative phenotype. The neonate had severe HDFN, anemia, and hyperbilirubinemia treated with exchange transfusion, intravenous immune globulin therapy, and phototherapy. It is interesting to note that in two of four reported cases, there was no clinical evidence of HDFN. Complement cascade proteins were found on RBCs in only one of these cases.¹¹

We discuss a blocking phenomenon of anti-K at a titer of 32, which is much lower than previously reported. The findings presented in our case are consistent with the blocking of K antigen sites by a potent anti-K bound on the surface of neonatal RBCs. Unlike most of the previous reports, anti-K

activated the complement cascade, probably because of the antibody's potency. Further studies are needed to verify this proposal.

A complete blockage of K antigens is not widely described or perhaps recognized. False-negative and false-positive phenotyping results with mono- and polyclonal antisera can occur with icteric, lipemic, or contaminated samples. Manufacturer product inserts do note this possibility. Laboratory staff and all other medical personnel involved in the diagnosis and treatment of HDFN need to consider the possibility of false-negative antigen results when performing phenotyping in the setting of a positive DAT, positive eluate, and positive IAT.

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