Immunohematology Case Studies 2019 - Blocked K antigen

Mirela Raos
Head of Transfusion Medicine Division
Clinical Department of Transfusion Medicine and Transplantation Biology
University Clinical Hospital Zagreb, Croatia
mraos@kbc-zagreb.hr

Clinical History

- A male neonate was born from the mother's fifth pregnancy in the 38th week of gestation
- Growth retardation was noticed and the pregnancy was completed because of CTG decelerations and oligohydramnios
- At delivery, the boy was 2410 g, 46 cm and the Apgar score was 9/10
- The second day after birth he required red cell transfusion because of significant anemia

Clinical History

Mother

- First pregnancy: healthy female neonate
- Second and third pregnancy: miscarriage, one in the 12th and the other in the 16th week of pregnancy
- Fourth pregnancy: healthy female neonate
- She never received any blood products

Serologic History

No antibody screening tests were preformed for any of her pregnancies

According to available data, she received pre-natal care, but there is no record of her ever undergoing antibody screening test

This is by no means standard practice in Croatia and can only be explained as an exception and a mistake

Current Sample Presentation Data

Mother

ABO/D: A, D positive, K negative

Antibody Screen Method: Indirect Antiglobulin Test (IAT) using Column Agglutination Technology (CAT) polyspecific (Biovue, Ortho Clinical Diagnostics)

Antibody Screen Results: Positive

Antibody Identification Method: IAT using CAT-Polyspecific and Neutral (Biovue, Ortho Clinical Diagnostics)

Antibody Identification Preliminary Results: anti-K in IAT (3+) and in enzyme-neutral test (2+), the autocontrol is negative

Current Sample Presentation Data

Neonate

ABO/D: AB D positive

DAT: positive (IgG, C3d)

Eluate (acid elution): (IAT using CAT): anti-K

K negative (K-k+)

Father

K positive (K+k+)

Antibody identification panel

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT	Enz
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	0
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	0	0
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	0
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	0	0
5	0	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	3+	2+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	3+	2+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	0	0
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0	0
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	0
AC																				0	NT

Alloantibody in mother's plasma

DAT on neonate's RBC

IgG	2+
C3d	1+
Control	0

Heel-stick sample

Eluate from the neonate's RBC

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT	
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	0	
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	0	
5	0	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	0	
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	3+	
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	3+	
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	0	
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0	
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	

Challenge with the Current Presentation

- DAT of the neonate is positive with IgG and C3d
- There is an anti-K in the eluate from the neonate's RBCs and in the mother's plasma
- The mother's RBCs were typed as K negative
- The father's RBCs were typed as K positive
- Initial review of results suggest RBCs of the neonate were blocked by potent maternal anti-K antibodies causing them to be negative when typing with anti-K reagent and anti-K antibody is causing HDFN

Interim Antibody Identification Possible Answers and Next Steps

- The neonate's RBCs appear to be K negative, but it seems fetal RBCs were blocked with a potent mother's anti-K antibody, which caused a negative result with the anti-K reagent
- Further testing, particularly molecular diagnostics, is needed for a conclusion

Further Work

- The K typing of the neonate's RBCs was repeated with another monoclonal anti-K reagent (Diaclone MS-56) and the result was negative
- Subclasses of anti-K were tested using DAT IgG1/IgG3 card and positive results were obtained with total anti-IgG (1:10) and -IgG_1 (1:100)
- The titer of anti-K in mother's sera in the tube test after delivery was 32 (score 37)

RBCs		1	2	4	8	16	32	64	Titer	Score
K+K+	RT	neg							0	0
	37°C	neg							0	0
	AHG	3+	2+	1+s	1+	1+	w	neg	32	37

Updated Clinical Information

- Soon after the first testing, the neonate was transfused with a K-negative red cell unit and repeated K typing wasn't reliable
- The infant’s anemia persisted and required further red cell transfusion on two more occasions
- He also had mild hyperbilirubinemia, which was treated with phototherapy

Updated Clinical Information

Laboratory parameters

Age of neonate	E ($x10^{12}$ /L)	Hb (g/L)	Hct (L/L)	Rtc (%%)	Bilirubin (µmol/L)
1st day	2.06	79	0.24	/	/
2nd day	2.22	78	0.25	82.9	112
2nd day (after 70 mL of RBCs)	4.03	128	0.39	51.4	109
3rd day	4.29	136	0.41	56.4	89
6th day (after this blood count finding, 50mL of RBCs was given and neonate was dismissed from hospital)	3.48	103	0.32	39.8	/
1 month	3.27	88	0.26	7.9	/
1 month and 4 days (after 50mL of RBCs)	4.91	132	0.39	7.1	27
2 months	3.81	106	0.31	/	/
5 months	5.27	139	0.39	/	/
Normal ranges	3.9-5.5	136-199	0.391-0.585	20-60	3-20

Further Testing Results and Interpretations

- The neonatal sample was also tested with PCR-SSP (Inno-train's RBC-Ready Gene kit) and was predicted to be K+k+
- Repeated typing was performed three months after the last transfusion, when DAT became negative, and showed that the infant was K positive

Conclusions

- The findings presented in this case are consistent with blocking of K antigen sites by a potent anti-K bound on the surface of the neonate's RBCs
- Anti-K in a titer of 32 and the IgG_1 subclass in a titer of 1:100 was causing severe HDFN that needed interventions: earlier completion of pregnancy due to CTG decelerations and oligohydramnios, red cell transfusions due to anemia and phototherapy due to hyperbilirubinemia

Blocked erytrocyte antigens

- Blocked erythrocyte antigens, first described in 1944, occurred due to potent maternal anti-D antibodies that blocked D antigens on fetal RBCs, causing them to be negative when typing with human immunoglobulin M anti-D. This phenomenon is rare and where it does occur, the antibody doesn't have to be of a high titer.
- There are only two case reports in literature that describe false negative K typing due to blocking with maternal anti-K. One study[1] showed that potent anti-K sera at a level of 256 or greater were capable of blocking the K antigen on K-positive cells. Another case reported a blocking phenomenon at an anti-K titer of 128[2].

Summary of Case Challenges

- Positive DAT of the neonate due to anti-IgG, and -C3d
- Anti-K was detected in the eluate
- The neonate's RBCs were typed as K negative
- Repeated typing with the monoclonal reagent from another manufacturer came out negative
- Anti-K was detected in the mother's plasma
- The mother's RBCs were typed as K negative and the father's as K positive
- PCR-SSP predicted the neonate to be K+k+
- Repeated typing 3 months after the last RBC transfusion confirmed the neonate to be K-positive

Lessons Learned by the Case

- In severe HDFN, the mother's anti-K antibodies can bind and block K antigen sites on the neonate's RBCs
- RBCs with a positive DAT can block K antigen sites and cause false negative results of K typing
- Complete blocking of the K antigen with the mother's anti-K antibodies is not widely described, nor perhaps recognized
- Manufacturers' product inserts should note this possibility and laboratory staff should also have this situation in mind when performing such tests
- The blocking effect of anti-K at the titer of 32 in this case is lower than previously reported

References

1. Lee E, Redman M, Owen I. Blocking of fetal K antigens on cord red blood cells by maternal anti-K. Transfusion Medicine 2009;19:139

2. Hannon J, Clarke G, Caruk B, Button E. Blocking phenomenon due to Anti-Kell in post-natal investigation. Transfusion Medicine 2007;17(4):ABS26