Immunohematology Case Studies 2020 - 1

Lilian Castilho
Hemocentro Unicamp, Campinas, SP, Brazil
Hospital Israelita Albert Einstein, São Paulo, Brazil
castilho@unicamp.br

Clinical History

- 24- year-old male
- African descent
- Diagnosis: Sickle Cell Anemia
- Transfused with 2 red cell units at another hospital over 3 months ago
- Ordered 1 red cell unit in our hospital
- Hemoglobin: 8 g/dL, Hematocrit=23%

Serologic History

The following serological information was provided:

- ABO/Rh: Group O/RhD+
- DAT: Negative
- Antibody screen: Positive with the 3 cells tested
- Antibody identification: panaglutination with negative autocontrol

- Sample referred to reference laboratory for additional testing

Current Sample Presentation Data

Results in our reference laboratory

- ABO/Rh: Group O/RhD+
- DAT: negative
- Antibody Screen Method: LISS-IAT and enzyme treated RBCs using gel agglutination (Grifols)
- Antibody Screen Results: positive 2+ with all 3 cells tested in LISS-IAT and 3+ in Papain treated RBCs
- Antibody Identification Method: LISS-IAT and Papain treated RBCs in gel agglutination (Grifols)
- Antibody Identification Results: reactive 2+ in LISS-IAT and 3+ in Papain treated RBCs with all cells. Negative autocontrols

Panel identification

IDENTISERA / IDENTISERA DIANA P

	RhPhenotype/Fenótipo Rh		Rh-hr						Kell				Duffy		Kidd		Lewis		P1	MNS				Luth	Xg	special antigens	Results	
			D	C	E	c	e	C^w	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Xg^a		Liss	Enz
1	CCDee	R_1R_1	+	+	0	0	+	0	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+		2+	3+
2	Ccddee	r´r	0	+	0	+	+	0	0	+	0	nt	0	+	0	+	+	0	+	0	+	0	+	0	+		2+	3+
3	ccDee	R_or	+	0	0	+	+	0	0	+	0	nt	+	+	+	0	0	+	+	+	0	+	0	0	+		2+	3+
4	ccddEe	r´´r	0	0	+	+	+	0	0	+	+	nt	0	+	+	+	+	0	+	+	+	+	+	0	0	Co(b+)	2+	3+
5	ccDEE	R_2R_2	+	0	+	+	0	0	0	+	0	nt	+	+	0	+	0	+	+	+	+	+	0	0	0		2+	3+
6	C^wCDee	$R_1{}^wR_1$	+	+	0	0	+	+	0	+	0	nt	0	+	+	0	0	+	+	+	+	0	+	0	+		2+	3+
7	ccddee	rr	0	0	0	+	+	0	0	+	0	nt	0	+	0	+	0	0	0	+	0	+	+	0	+		2+	3+
8	ccddee	rr	0	0	0	+	+	0	0	+	+	nt	0	+	+	0	+	0	+	+	0	+	0	0	+		2+	3+
9	ccddee	rr	0	0	0	+	+	0	0	+	0	nt	+	0	+	0	0	+	+	0	+	0	+	0	+		2+	3+
10	ccddee	rr	0	0	0	+	+	0	0	+	0	0	+	0	0	+	0	+	+	0	+	+	+	0	+		2+	3+
11	CCDee	R_1R_1	+	+	0	0	+	0	0	+	0	nt	0	+	0	+	0	+	0	+	0	+	0	+	0		2+	3+

Similar strength of antibody reactivity with all cells associated with negative DAT and autocontrols suggest the presence of an alloantibody to a high prevalence antigen resistant to papain

Further work

Selected cell panel using, LISS, papain, **α-Chymotrypsin** and **DTT**:

SERASCAN DIANA 4 / SERASCAN DIANA 4P/ SERASCAN DIANA Di^a

	RhPhenotype/Fenótipo Rh		Rh-hr						Kell				Duffy		Kidd		Lewis		P1	MNS				Luth	Xg	special antigens	Results		DTT	α-Chym
			D	C	E	c	e	C^w	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Xg^a		Liss	Enz		
I	CCDee	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	nt	0	+	+	+	0	+	0	+	0	+	+	0	0		2+	3+	0	0
II	ccDEE	R_2R_2	+	0	+	+	0	0	0	+	0	nt	+	+	0	+	+	0	+	0	+	0	+	0	+		2+	3+	0	0
III	ccddee	rr	0	0	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+		2+	3+	0	0
IV	ccddee	rr	0	0	0	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	+	+	0	0	+		2+	3+	0	0
Di^a	CcDdee	rr	0	0	0	+	+	0	0	+	0	0	+	0	+	+	0	+	+	0	+	+	0	0	+	Di(a+)	2+	3+	0	0
Autocontrole																											0	0	-	-

DTT and α-Chymotrypsin-treated RBCs were non-reactive

Effect of proteases and thiol reagents on selected antigens

Ficin/Papain	Trypsin	α-Chymotrypsin	DTT/2ME/AET	Antigen
Sensitive	Sensitive	Sensitive	Resistant	Bp^a; Ch/Rg; XG
Sensitive	Sensitive	Sensitive	Sensitive	IN; JMH
Sensitive	Sensitive	Resistant	Resistant	M, N, En^{a}TS; Ge2, Ge4
Sensitive	Resistant	Sensitive	Resistant	'N'; Fy^a, Fy^b
Variable	Resistant	Sensitive	Resistant	S, s
Variable	Resistant	Sensitive	Weakened or Sensitive	YT
Sensitive	Resistant	Resistant	Resistant	En^{a}FS
Resistant	Sensitive	Sensitive	Weak or Sensitive	LU, MER2
Resistant – Papain	Sensitive	Sensitive	Sensitive	KN
Weakened or sensitive – Ficin				
Resistant	Sensitive	Weakened	Sensitive	DO
Resistant	Resistant	Sensitive	Weakened	CROM

Most likely Blood Group Systems implicated: YT, LU, KN, DO, CROM
Antigens DTT and **α-Chymotrypsin** sensitive and **Papain** resistant

Genotyping

ID-CORE XT platform (Grifols)

Sistema de grupo sanguíneo	Alelos analisados	Resultado de genótipo	Antígenos (ISBT)	Resultado de fenótipo previsto
Rh	RHCE*ce RHCE*Ce RHCE*cE RHCE*CE RHCE*CeCW RHCE*ceCW RHCE*CECW RHCE*ceAR RHCE*CeFV RHCE*CeVG RHCE*cEFM RHCE*ce[712G] RHCE*ce[733G] RHCE*Ce[733G] RHCE-D[5, 7]-CE RHCE*ce[733G,1006T] RHCE*cE[712G,733G] RHD*r's-RHCE*ce[733G,1006T]	RHCE*ce, RHCE*Ce	C (RH2)	+
			E (RH3)	0
			c (RH4)	+
			e (RH5)	+
			CW (RH8)	0
			V (RH10)	0
			hrS (RH19)	+
			VS (RH20)	0
			hrB (RH31)	+
Kell	KEL*K_KPB_JSB KEL*k_KPB_JSB KEL*k_KPA_JSB KEL*k_KPB_JSA	KEL*k_KPB_JSB	K (KEL1)	0
			k (KEL2)	+
			Kpa (KEL3)	0
			Kpb (KEL4)	+
			Jsa (KEL6)	0
			Jsb (KEL7)	+
Kidd	JK*A JK*B JK*B_null(871C) JK*B_null(IVS5-1a) JK*A_null(IVS5-1a)	JK*A, JK*B	Jka (JK1)	+
			Jkb (JK2)	+
Duffy	FY*A FY*B FY*B_GATA FY*B[265T]_FY*X FY*A[265T]_FY*X FY*A_GATA	FY*A, FY*B	Fya (FY1)	+
			Fyb (FY2)	+
MNS	GYPA*M GYPA*N	GYPA*N	M (MNS1)	0
			N (MNS2)	+
	GYPB*s GYPB*S GYP.Mur GYPB*deletion GYPB*S_null(230T) GYPB*S_null(IVS5+5t)	GYPB*s	S (MNS3)	0
			s (MNS4)	+
			U (MNS5)	+
			Mia (MNS7)	0
Diego	DI*A DI*B	DI*B	Dia (DI1)	0
			Dib (DI2)	+
Dombrock	DO*A DO*B DO*B_HY DO*A_JO	DO*B	Doa (DO1)	0
			Dob (DO2)	+
			Hy (DO4)	+
			Joa (DO5)	+
Colton	CO*A CO*B	CO*A	Coa (CO1)	+
			Cob (CO2)	0
Cartwright	YT*A YT*B	YT*A	Yta (YT1)	+
			Ytb (YT2)	0
Lutheran	LU*A LU*B	LU*B	Lua (LU1)	0
			Lub (LU2)	+

Genotyping Results

Genotype: *RHCE*ce/RHCE*Ce, KEL*2/KEL*2, KEL*4/KEL*4, KEL*7/KEL*7, JK*1/JK*2, FY*1/FY*2, GYPA*2/GYPA*2, GYPB*4/GYPB*4, GYPB*5, DI*B/DI*B, DO*2/DO*2 , DO*4, DO*5, CO*1/CO*1, LU*2/LU*2*

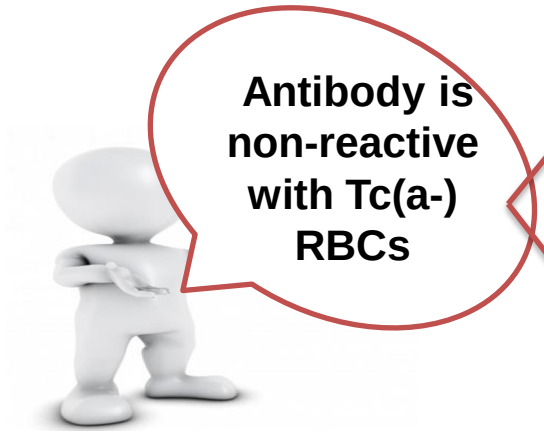
Predicted phenotype : C+E-c+e+, K-k+, Kp(a-b+), Js(a-b+), Jk(a+b+), Fy(a+b+), M-N+,S-s+, U+, Mi(a-), Di(a-b+), Do(a-b+), Hy+, Jo(a+), Co(a+b-), Yt(a+b-), Lu(a-b+)

No negative results for high prevalence antigens in DO, YT and LU systems

Further Antibody Investigations

High prevalence antigen negative RBCs tested in a selected cell panel (Tc^a, Hy, Jo^a, Gy^a, Yt^a, Lu^b, Kn^a)

D	C	E	c	e	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	U	Tc^a	Hy	Jo^a	Gy^a	Yt^a	Lu^b	Kn^a	LISS	ENZ
+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	+	-	-	-	-	-	-	2+	3+
+	+	+	+	+	0	0	0	+	0	+	+	0	+	+	0	-	-	-	-	-	-	0	0
+	0	0	+	+	0	0	0	+	0	+	+	+	+	+	-	-	-	-	-	-	-	2+	3+
+	0	0	+	+	0	0	0	+	+	+	+	0	+	+	-	-	-	-	-	-	-	2+	3+
+	+	0	0	+	0	0	+	+	0	+	0	+	+	+	-	0	0	0		+	+	2+	3+
+	+	0	+	+	0	+	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	2+	3+
0	0	0	+	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	+	0	+	2+	3+
+	+	0	+	+	0	+	0	0	+	+	+	0	+	+	+	+	+	+	+	+	0	2+	3+



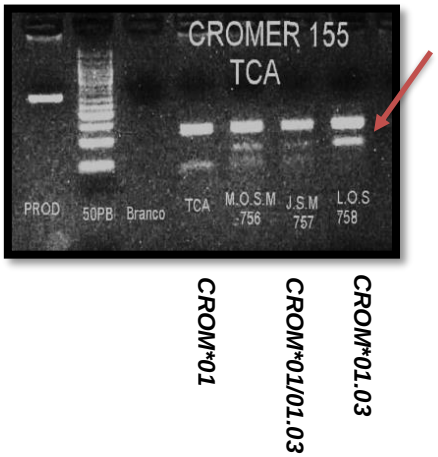
	Papain	α-Chymo	DTT
YT	+/-	-	+/-
DO	+	+/-	-
CROM	+	-	+/-
JMH	-	-	-
PATIENT	+	-	-

Tc^a (CROM2) Phenotyping and Genotyping

Results

- Phenotyping performed with one anti-Tc^a from our rare serum collection and one anti-Tc^a kindly provided by Dr Thierry Peyrard showed negative results with the patient RBCs (CROM:-2,3)

- Genotyping performed by PCR-RFLP on DNA sample from the patient confirmed phenotyping results



Patient sample

Further Serological Work

- Allogeneic adsorptions were performed in order to rule out additional common alloantibodies hidden by the alloantibody to a high prevalence antigen

- Three cells were employed:
  R_2R_2, K-, S-
  R_1r, K+, S-
  R_1r, K-, S+

- Allogeneic adsorptions: After the third adsorption the samples were tested in the panel

- Results of the allogeneic adsorption
  - Panel with three times-adsorbed serum with the 3 cells removed the high prevalence antibody and it was possible to rule out anti-E, -K and -S

Next Steps

The patient serum was crossmatched with RBCs from his siblings

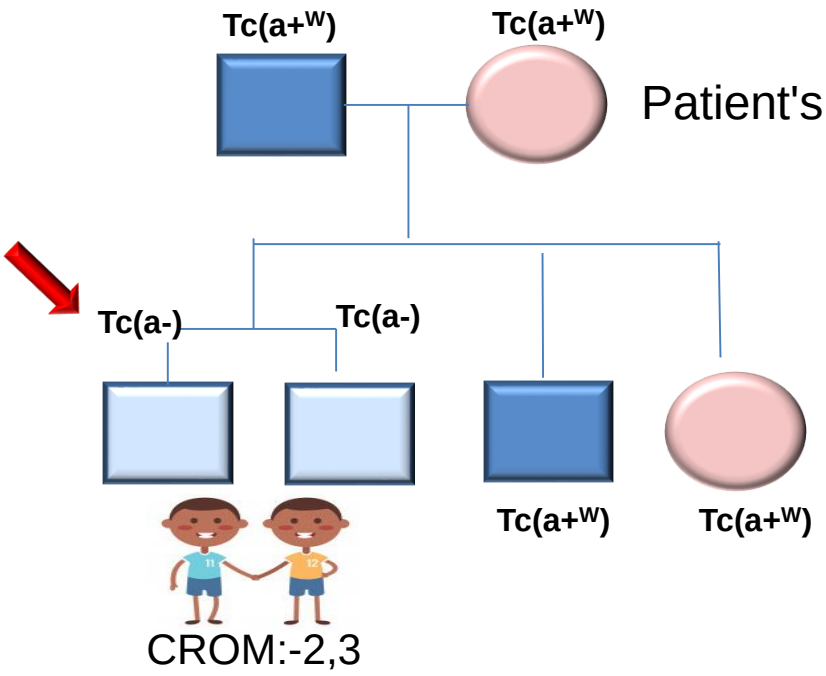
Crossmatch results

SIBLINGS		GEL TEST	
		LISS-IAT	PAPAIN
Father	O-	0	0
Mother	O+	Weak	0
Sister	O+	0	0
Brother	O+	0	0
Twin	O+	0	0

Serological results were compatible with all sibling's RBCs but not his mother's RBCs with a weak reaction in LISS-IAT

Further Work

Tc^{a} (CROM2) phenotyping results of the patient's siblings



Only the proband and his twin were Tc(a-)
RBCs from the other siblings showed a weak reactivity with anti-Tc^{a}

Further Work

CROM2 genotyping results of the patient's siblings

Siblings	CROM2 Genotypes	Predicted phenotypes
Father	*CROM*01/CROM*01.03*	Tc(a+b+) or CROM:2,3
Mother	*CROM*01/CROM*01.03*	Tc(a+b+) or CROM:2,3
Sister	*CROM*01/CROM*01.03*	Tc(a+b+) or CROM:2,3
Brother	*CROM*01/CROM*01.03*	Tc(a+b+) or CROM:2,3
Twin	***CROM*01.03/CROM*01.03***	Tc(a-b+) or CROM:-2,3
Patient	***CROM*01.03/CROM*01.03***	Tc(a-b+) or CROM:-2,3

Genotype results showed that only the proband and his twin were *CROM*01.03* homozygous. The other siblings were heterozygous confirming the phenotyping results.

Conclusions

- Patient's serum contains an IgG antibody to the high prevalence Tc^a (CROM2) antigen confirmed by serological and molecular studies
- Tc^a (CROM2) phenotyping showed that this antigen is weakly expressed in Tc(a+b+) heterozygous cells
- As anti-Tc^a (CROM2) can be implicated in transfusion reaction, Tc^a (CROM2) phenotyping and/or genotyping should be performed before the release of the compatible red blood cell unit

Summary of Case Challenges

- This case represents a rare example of anti-Tc^{a} (CROM2) sensitive to DTT200mM, reactive with Tc(a+b-) homozygous cells but weak or non-reactive with Tc(a+b+) heterozygous cells identified in a Sickle Cell Disease patient
- Siblings of the patient were tested for compatibility but only his twin was compatible. Unfortunately he has also sickle cell anemia and can not donate blood to the patient
- This case reminds that crossmatch testing with a low-titer antibody can be negative on heterozygous cells, while the antibody can be detectable upon an antibody screening test
- The patient has not yet been transfused and is being monitored
- MMA has been suggested to assess the clinical significance of the antibody

- High prevalence antigen negative individuals may result from consanguineous marriages
- The use of different enzymes and thiol reagents is very helpful in the identification of antibodies to high prevalence antigens
- Antibodies occasionally do not behave as expected
- For very rare specificities, the characteristics described are based on limited observations
- Use the features described as a GUIDE and not as a RULE
- The correct determination of an antibody requires competence in manual serological techniques and molecular tests

ISBT Terminology of the System

Cromer Blood Group System

- ISBT symbol: CROM (021)
- Gene name: *CROM*
- ISBT symbol (number) for Tc^{a} antigen : CROM2 (021002 0r 21.2)
- The reference allele is *CROM*01* encoding CROM2
- Tc(a-b+) or CROM:-2,3: allele name is *CROM*01.03*

Cromer Blood Group System

- Cromer blood group system consists of 17 antigens carried on a GPI-linked glycoprotein (DAF, CD55) that consists of 481 amino acids.
- The system was named after the first antigen in the system, Cr^a
- The gene encoding Cromer antigens is named *CROM (DAF),* located in Chromosome 1q32.2 and organized in 11 exons distributed over 40kbp of gDNA
- The reference allele is *CROM*01*
- Tc^a (CROM2) is a high frequency antigen with an occurrence of 100% in most populations and >99% in Blacks.
- Antithetical antigens: Tc^b (CROM3) and Tc^c (CROM4)
- All Tc(a-) Blacks are Tc(b+); Tc(a-) Caucasians are Tc(c+)
- The molecular basis of CROM:-2,3 is c.155G>T in exon 2 (p.Arg52Leu)
- Anti-Tc^a (CROM2) is a rare antibody, IgG and not involved in HDFN but can be implicated in transfusion reactions

References

1. Kowalski MA et al. Hemolytic transfusion reaction due to anti-Tc(a). Transfusion 1999; 39:948-950

2. Lublin DM. et al. Molecular basis of Cromer blood group antigens. Transfusion 2000; 40:208-213

3. Storry JR, Reid ME, Yazer MH. The Cromer blood group system: a review. Immunohematology 2010; 26:109-118.

4. Daniels GL. Human Blood Groups, 3rd ed. Oxford, UK: Wiley-Blackwell, 2013.

5. Reid ME, Lomas-Francis C, Olsson ML. The Blood Group Antigen FactsBook, 3rd ed. San Diego, CA, Elsevier, 2012.

Website:

International Society of Blood Transfusion (ISBT) - www.isbtweb.org