Immunohematology Case Studies 2016 - 6

Dr. Eduardo Muñiz-Diaz, MD, PhD
Head of the Immunohaematology Division
Blood and Tissue Bank
Barcelona, Spain
emuniz@bst.cat

Clinical History

- 27-year old female in the 30th week of her second pregnancy belonging to the Romani (Gypsy) ethnic group.
- In the first pregnancy she had delivered a preterm child by Caesarean section due to placental ablation and the critical condition of the foetus.
- A presumed antibody to a high frequency antigen was detected in her plasma and a sample was sent to the Immunohematology Reference Laboratory of the Blood and Tissue Bank in Barcelona, Spain.

Serologic History

Hospital Transfusion Laboratory results

The following serological information was provided:

ABO/Rh: Group O, D+

DAT: Negative

Antibody Screen Results: Positive with the 3 cells (4+)

Antibody Identification Preliminary Results: Positive with all cells (4+) in IAT with untreated and enzyme treated cells.

Serologic History

Reference Laboratory Results

The results obtained in the hospital were confirmed.

- Group O, D+
- DAT: Negative
- Antibody Screen Method: IAT using Column Agglutination Technology (CAT) and also LISS tube IAT
- Antibody Screen Results: Positive with all cells
- Antibody Identification Methods: LISS tube IAT and IAT using CAT with untreated and enzyme (papain) treated cells
- Antibody Identification Results: Positive with all cells using tube and column in IAT with untreated (4+) and enzyme treated cells (4+).
- In addition we also confirmed that the autocontrol was negative.

Panel Sample

		Rh						Kell						Duffy		Kidd		Lewis		P	MNS				Luterano		Xg^a		Results			
		D	C	E	c	e	CW	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Lu^a	Lu^b	Xg^a		IAT	Enz		
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	+	0	+	0	1	4+	4+		
2	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	+	0	+	0	+	+	2	4+	4+		
3	R_2R_2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	0	3	4+	4+		
4	R_1r	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	4	4+	4+		
5	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	0	+	0	5	4+	4+		
6	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	6	4+	4+		
7	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	0	+	0	7	4+	4+		
8	R_0r	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	+	8	4+	4+		
9	r′ r	0	+	0	+	+	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	9	4+	4+		
10	rr′′	0	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+	10	4+	4+		
11	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	11	4+	4+		
Patient																												0	0		

The strength and consistency of reactivity with all cells,
the DAT and the autocontrol negative made very likely the diagnosis of an alloantibody to a high frequency antigen

Further Work

- There were not transfusion antecedents
- Patient extended phenotype was studied:

1. The extended phenotype for common red blood cell antigens was:

   D+C+c+E-e+ (R_1r) K- k+ Kp(a-b+) Jk(a+b+) Fy(a-b+) M+ N- S+s+

2. Other rare high frequency antigens were investigated with the following results:

   Jr(a+) Vel+ Di(b+) Lan+ Ge3+ PP1P^k+ Kn(a+) Sl(a+) Cr(a+) P+ Yt(a+) Er(a+) Co(a-)

3. The Colton phenotype was repeated and Co^b typing was also included: Co(a-b-)

Genotyping Results

READY GENE Rare ID IVD CE LOT S9SP080

Protocol for Documentation 2012-01

Date: 23-8-11

Primermix No.	1	2#	3	4	5	6	7*	8	9	10	11	12	13	14#	15	16
PCR-Product (size in bp)	166	165	184	184	207	207	193	195	218	216	197	197	213	214	181	179
Antigen	**Kp**		**Lutheran**		**Diego**		**Wright**		**Cartwright**		**Colton**		**Knops**		**Dombrock**	
Serological name	Kpa	Kpb	Lua	Lub	Dia	Dib	Wra	Wrb	Yta	Ytb	Coa	Cob	Kna	Knb	Doa	Dob
Allele	KEL 3	KEL 4	LU1	LU2	DI1	DI2	DI3	DI4	Y1	Y2	CO1	CO2	KN1	KN2	DO1	DO2
Example for result	-	+	-	+	-	+	-	+	+	-	+	-	+	-	+	+
	Kp bb		Lu bb		Di bb		Wr bb		Yt aa		Co aa		Kn aa		Do ab	
Result	−	+	−	+	−	+	−	+	+	−	+	−	+	−	+	+

Homozygosity for the *CO*01* allele was observed: Co^a Co^a genotype

An apparent discrepancy between phenotyping and genotyping results was found

Further Phenotyping Work

- If genotyping results were correct the predicted phenotype should be Co(a+b-).

- To resolve this (apparent) discrepancy between the phenotype and the genotype, the Colton phenotype was repeated including two additional examples of anti-Co^{a} and anti-Co^{b} and one example of anti-Co3.

	Anti-Co^{a}	Anti-Co^{a}	Anti-Co^{b}	Anti-Co^{b}	Anti-Co3
Patient's red blood cells	0	0	0	0	0

Again, the results were compatible with a Co (a-b-) phenotype and the suspicion that an anti-Co3 was present in the pregnant woman's plasma was very appealing.

Further Serological Work

- To exclude the presence of additional alloantibodies:

1. Allogeneic adsorptions were performed in order to exclude the presence of additional common alloantibodies hidden by the alloantibody to a high frequency antigen.

Allogeneic adsorptions were performed with two cells carrying a complementary phenotype for the most common red cell antigens:

1. R1R1, K-, Jk(a+b-), Fy(a-b+), SS
2. rr, K-, Jk(a-b+), Fy(a+b-), ss

Adsorptions (x3) were made with a PEG method

Further Serological Work

Results of the allogeneic adsorption procedure

		Rh						Kell						Duffy		Kidd		Lewis		P	MNS				Luterano		Xg^a		Results			
		D	C	E	c	e	CW	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Lu^a	Lu^b	Xg^a		IAT	Enz	ADS 1	ADS 2
1	$R_1^WR_1$	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	+	0	+	0	1	4+	4+	0	0
2	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	+	0	+	0	+	+	2	4+	4+	0	0
3	R_2R_2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	0	3	4+	4+	0	0
4	R_1r	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	4	4+	4+	0	0
5	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	0	+	0	5	4+	4+	0	0
6	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	6	4+	4+	0	0
7	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	0	+	0	7	4+	4+	0	0
8	R_0r	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	+	8	4+	4+	0	0
9	r′ r	0	+	0	+	+	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	9	4+	4+	0	0
10	rr′′	0	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+	10	4+	4+	0	0
11	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	11	4+	4+	0	0
Patient																												0	0	0	0

Other alloantibodies against common antigens hidden by the high frequency alloantibody were excluded in the two adsorbed sera

Further Serological Work

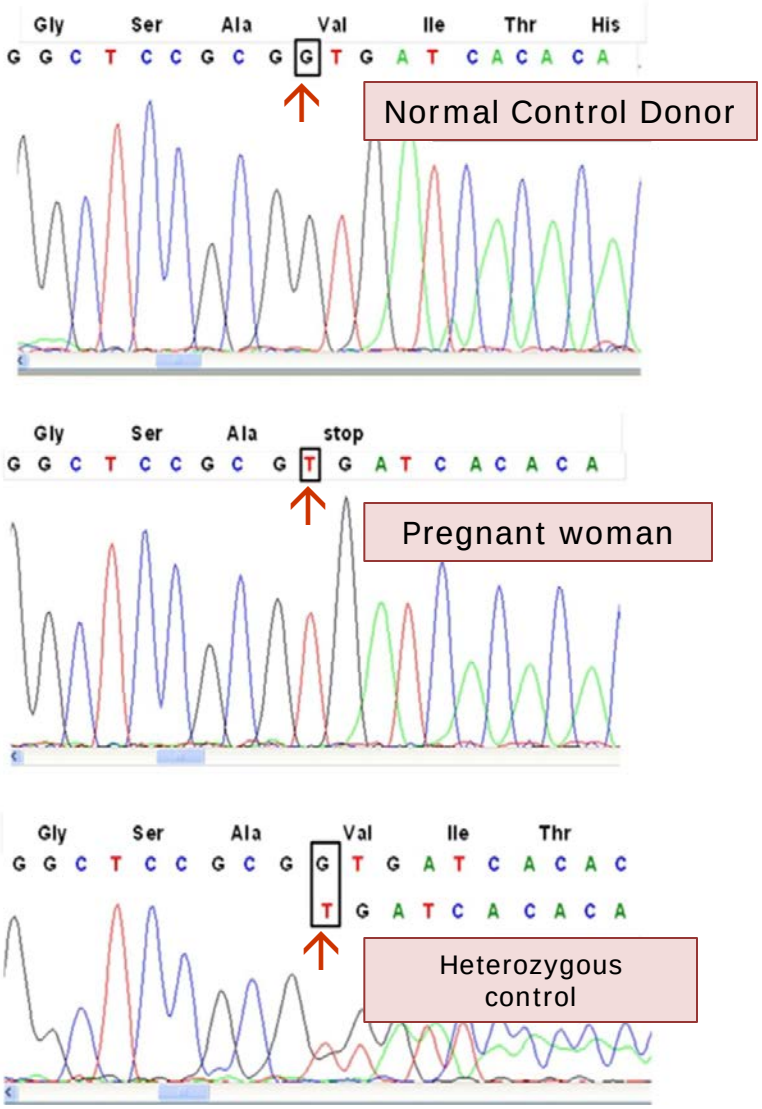
2. To confirm the presence of anti-Co3 in this woman her unabsorbed plasma was faced again a selection of three cells with different Colton phenotypes.

	Co(a-b+)	Co(a+b-)	Co(a-b-)
Woman's plasma	++++	++++	0

Positive results were obtained when Co^a or Co^b antigens were present, but negative results were obtained when both were absent ($Colton_{null}$ phenotype).

Serological results were compatible with the presence of anti-Co3 in a woman carrying a $Colton_{null}$ phenotype

Further Genotyping Work



- To resolve the discrepancy between the Co(a-b-) phenotype and the *CO*01 genotype*, DNA sequencing of the *AQP1*, exon 1 and the *AQP1*, exons 2-4 specific amplicons was performed.

- A homozygous single nucleotide deletion within exon 3 was observed. An *AQP1g.15763delG* mutation corresponding to an *AQP1c.601delG* mutation of the cDNA induced a frameshift with a premature stop within the next codon n.201.

Figure courtesy of Dr BK Flesch

The Colton system
History

- In 1965 in Oslo (Norway), the discovery of an antibody that detected a “public” antigen was linked to two other cases discovered earlier by workers in Minneapolis, Oxford, and London.

- The antigen was named Co^a (Colton) in 1967 for the first of the three producers of anti-Co^a; it should actually have been named “Calton” but the hand writing on the tube apparently was misread.

- In 1970 the antithetical antigen, Co^b, was reported.

- In 1974 the Co(a-b-) phenotype of three persons in a French Canadian family was reported. The serum from a patient in 1964 (Swarts) was eventually found to be negative only with these family members.

- Colton is the 15^{th} human blood group system recognized by the ISBT (ISBT 015).

- The Colton blood group antigens are encoded by the *AQP1* gene which produces a water channel forming integral protein.

The Colton system

Molecular basis of the Co(a-b-) phenotype

- Different mutations leading to reduced or even abolished expression of the Colton blood group on red cells have been demonstrated.

- These mutations are based on either single nucleotide exchange or result from frameshifts due to the insertion or deletion of single nucleotides.

- The *AQP1 del601G* mutation was previously described in a French “gypsy woman” of Spanish origin. And in addition to the case presented here, the same mutation was also reported in three more patients belonging to the Romani (Gypsy) ethnic group, one of them also of Spanish origin.

- These reports suggests an evident accumulation of this particular *AQP1* mutation, among the small number of mutations of the gene, in Romani (Gypsy) patients, especially in those of Spanish origin.

- In an estimated population of 700,000 Romani in Spain, there could be some thousand carriers of the mutation. Due to a higher percentage of consanguinity in closed societies, an important number of homozygous carriers resulting in a Co-negative phenotype is very likely. The parents of our patient were cousins.

The Colton system

Phenotypes (% occurrence) and Clinical Significance

Phenotype	% Occurrence (most populations)
Co (a+b-)	91.4
Co (a-b+)	0.2
Co (a+b+)	8.4
Co (a-b-)	<0.01

- Few reports of significant delayed or acute transfusion reactions or haemolytic disease of the foetus and newborn (HDFN) due to anti-Co^{a} have been reported, although both are known to occur with severe morbidity.
- Anti-Co^{b} is relatively rare and usually not involved in transfusion reactions or significant HDFN.
- Anti-Co3 was reported to cause severe HDFN requiring neonatal transfusion. Transfusion of incompatible Co(a+b-) red cells in a patient with anti-Co3 was reported to be responsible for a mild transfusion reaction.

Further Work

To predict the fetal risk in this case

1. The treatment of the mother's plasma with 2-mercaptoethanol confirmed that the anti-Co3 was IgG.
2. Antibody's titration showed a titer of 4096.
3. Genotyping of the father showed a Co^a Co^a genotype indicating that the fetus would inherit the allele Co^a making feasible the reaction of maternal antibody to fetal erythrocytes.
4. Crossmatching between the mother's plasma and red cells from the father was very positive (4+)

Further Work

Searching for compatible blood

1. At that time, no Co(a-b-) blood was available from the International Panel on Rare Blood Donors and autologous donation was not possible.
2. Three of the mother's siblings were phenotyped for Co antigens.

	Co^a	Co^b	Co3	Phenotype
Brother 1	0	0	0	Co (a-b-)
Brother 2	0	0	0	Co (a-b-)
Sister	+	0	+	Co(a+b-)

3. The two brothers Co (a-b-) showed a negative crossmatch.

	Co (a-b-)	Co (a-b-)	Co (a+b-)
Maternal serum	0	0	++++

However they only accepted to give blood in case of a true complication during or after delivery...

Clinical and Serological data from the newborn

The baby was delivered at term with moderate anaemia and jaundice but recovered after 1 week under phototherapy without transfusion.

- The Child's blood groups were B, D+
- The direct antiglobulin-test (DAT) was Positive: 3+
- Elution was not performed in the hospital blood transfusion service where the mother gave birth

Conclusions

- Anti-Co3 (IgG, titer 4096) in a pregnant Gypsy woman carrying a Co(a-b-) phenotype was identified during her second pregnancy.

- An apparent discrepancy between phenotype Co(a-b-) and genotype (*Co^{a} Co^{a}*) was found.

- An *AQP1 c.601de*lG mutation was causative for the Co(a-b-) phenotype. The same mutation has also been found in four patients from different regions of Europe belonging to the Romani (Gypsy) ethnic group, two of them also of Spanish origin. There was no apparent relationship between the reported cases .

- No Co(a-b-) blood was available in the course of the pregnancy and delivery.

- The baby was delivered at term with moderate HDFN. He recovered after one week under phototherapy.

Lessons Learned by the Case

- Discrepancies between phenotype and genotype are common in the case of null phenotypes. Our woman had a Co (a-b-) phenotype but genotyping showed homozygosity for the *CO*01* allele (Co^a Co^a).

- When these discrepancies are not taken into account they can interfere in the correct antibody identification.

- Anti-Co3 was reported to cause severe HDFN requiring neonatal transfusion, but because of the rarity of the Co(a-b-) phenotype no compatible blood was available in the world at that moment.

- In these cases the patient's relatives, especially siblings, can be the best solution. In our case, two brothers with the same phenotype were located to donate blood, but they decided that would only give blood in the event that an unexpected complication in the mother occurred or in the case of severe fetal involvement.

- The high number of Roma people living in Spain makes predictable diagnosis of new cases of anti-Co3. In fact, two more cases in pregnant women have been diagnosed in our laboratory after the case presented.

References

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Immunohematology Reference Laboratory
Blood and Tissue Bank. Barcelona, Spain