Immunohematology Case Study 2016 - 3

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Clinical History

- Medical history:

A 33 years old female, pregnant at 25 weeks gestation

- Transfusion history:

No transfusions

- Pregnancy history:

1 previous pregnancy resulted in a live birth

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Serologic History

- The patient underwent routine blood work

- The referring hospital transfusion service reported that antibody screen and identification were positive with all cells, while DAT was negative

- Due to limited local resources, a sample was sent to Immunohematology Reference Laboratory at Policlinico Hospital of Milan (Italy) for further testing

Current Sample Presentation Data

- ABO/Rh: A Rh positive, ccEe, kk
- Direct Antiglobulin Test (DAT): negative
- Antibody Screen Method: Indirect Antiglobulin Test (IAT) using Column Agglutination Technology (CAT) polyspecific (Biovue, Ortho Clinical Diagnostics)
- Antibody Screen Results: all cells positive (score 2+)
- Antibody Identification Method: IAT using CAT-Polyspecific, polyethylene glycol (PeG), low-ionic-strength saline solution (LISS) tube and saline
- Antibody Identification Preliminary Results: all cells positive in IAT and negative in Saline

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Antibody Identification
Preliminary Results

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

AC: autocontrol

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Antibody Identification
Preliminary Results

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	LISS	S20
1	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	2+	0
2	+	+	0	0	+	+	0	+	+	0	+	0	0	+	0	+	+	0	+	2+	0
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	2+	0
4	+	0	+	0	+	0	0	+	0	0	+	0	+	0	+	+	+	0	0	2+	0
5	0	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	+	0	+	2+	0
6	0	0	+	+	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	2+	0
7	0	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	2+	0
8	0	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0	+	0	+	2+	0
9	0	0	+	0	+	0	0	+	0	+	+	+	+	0	0	+	+	0	+	2+	0
10	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	2+	0
11	0	0	+	0	+	0	0	+	0	+	+	+	0	0	+	+	+	0	+	2+	0
AC	+	0	+	+	+	0	0	+	0	+	+	+	0	+	+	+	0	+	+	0	0

AC: autocontrol

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Challenge with the Current Presentation

- All cells tested were positive, but the autocontrol was negative

- An alloantibody to a high-prevalence antigen was suspected since the strength and consistency of reactivity were uniform for all the cells tested

- Antibodies to a high-frequency antigen (HFA) can be identified by:

✓ performing an extended phenotype on the patient's red cells
✓ typing the patient's red cells with selected HFA antisera
✓ matching selected rare phenotype and null cells against patient's plasma
✓ testing reagent red cells matching with the patient's phenotype

Supplementary Tests:
Patient extended phenotype/genotype

Antigens	Serology	Antigens	Serology	Antigens	Molecolar Biology
C^w	0	Kp^b	+	M	+
K	0	Js^b	+	N	0
k	+	Lu^b	+	Di^a	0
Jk^a	+	$PP1P^k$	+	Di^b	+
Jk^b	+	U	+	Wr^a	0
Fy^a	0	Vel	+	Wr^b	+
Fy^b	+	Yt^a	+	Kn^a	+
S	+	Co^a	+	Kn^b	0
s	+	LAN	+	Do^a	+
Le^a	0	Ge	+	Do^b	0
Le^b	+	Jr^a	+	Do^a	+
P_1	+	Sc1	+	Do^b	0
		LW^a	+	Kp^a	0

Supplementary Tests:
Testing reagent red cells matching with the patient's phenotype

																			Test Results				
Donor cell code	**D**	**C**	**c**	**E**	**E**	**C^w**	**K**	**k**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**Le^a**	**Le^b**	**P_1**	**M**	**N**	**S**	**s**	**CAT**	**AHGT IgG**	**Ficin IgG**	**DTT IgG**	**α–Chemio tripsin IgG**
I042813020522	+	0	+	+	+	0	0	+	0	+	+	0	0	+	+	+	0	+	+	2+	1+	2+	0	1+

- Certain blood group antigens can be destroyed or weakened by chemical treatment of the cells

- The use of modified red cells can be especially helpful to identify an antibody to a HFA or to cell-surface CD38 protein

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Effect of enzyme treatment/chemical modification on antigens

Ficin/ Papain	Trypsin	α-Chymo-trypsin	200 mM DTT/AET	Possible specificity
Negative	Negative	Negative	Positive	Bp[a]; Ch/Rg; XG
Negative	Negative	Negative	Negative	IN; JMH
Negative	Negative	Positive	Positive	M, N, En[a]TS; Ge2, Ge4
Negative	Positive	Negative	Positive	'N'; Fy[a], Fy[b]
Variable	Positive	Negative	Positive	S, s
Variable	Positive	Negative	Weak or negative	YT
Negative	Positive	Positive	Positive	En[a]FS
Positive	Negative	Negative	Weak or negative	LU, MER2
Positive – Papain Weak or negative – Ficin	Negative	Negative	Negative	KN
Positive	Negative	Weak	Negative	DO
Positive	Positive	Negative	Weak	CROM
Positive	Positive	Negative	Positive	Some DI (3rd loop)
Positive	Positive	Positive/weak	Negative	LW
Positive	Positive/weak	Positive/weak	Positive	SC
Positive	Positive^	Positive^	Negative	KEL^ (except KALT, which is trypsin sensitive)
Positive	Positive	Positive	Positive	ABO; En[a]FR, U; P1PK; RH; LE; Fy3; JK; most DI; CO; H; Ge3; OK; Vi; P, FORS; JR; LAN, Cs[a]; ER; LKE, PX2; Vel,† ABTI; At[a]; Emm; AnWj; Sd[a]; PEL; MAM
Positive	Positive	Positive	Enhanced	Kx

^Kell blood group system antigens are sensitive to treatment with a mixture of trypsin and α-chymotrypsin.
†DTT may be variable.

- The patterns of reaction are a useful guide in antibody identification
- The possible specificity is limited to 4 systems: LW, SC, KEL, DO

Marion E. Reid, Christine Lomas-Francis and Martin L. Olsson.
The Blood Group Antigen. Facts Book - Third Edition - Elsevier

Supplementary Tests:

Testing selected red cells

Donor cell code	D	C	c	E	e	C^w	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	CAT
Ko	+	+	0	0	+	0	0	0	+	+	+	+	0	0	+	+	0	0	+	**2+**
Gy(a-) Donor 55193	+	+	+	+	+	/	0	+	0	+	+	0	0	+	+	0	+	0	+	**0**
Gy(a-) Donor VJ4156-213	0	0	+	0	+	0	0	+	0	+	+	+	/	/	/	+	+	+	+	**0**

- According to the results, we identified antibodies against Dombrock system
- Molecular typing for DO antigens was: Do(a+b-)
- We performed serology to confirm the molecular typing

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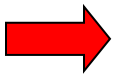
Supplementary Tests:

serology vs molecular

Patient's type for DO system by serology



Antigens	Molecular biology	Antigens	Serology
Do^a	+	Do^a	0
Do^b	0	Do^b	0
		Gy^a	0

We observed discrepancy between serology and molecular typing for DO system

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Supplementary Tests: Adsorption

- Antibodies to high-prevalence antigens may be accompanied by antibodies to common antigens
- It may be necessary to adsorb the antibody to the high-prevalence antigen onto red cells that express the corresponding antigen and are negative for patient's common antigens

Phenotype of adsorbing cell: A, ccDEE, C^w-, K-, Fy(a-)

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

No alloantibodies to common antigens were identified

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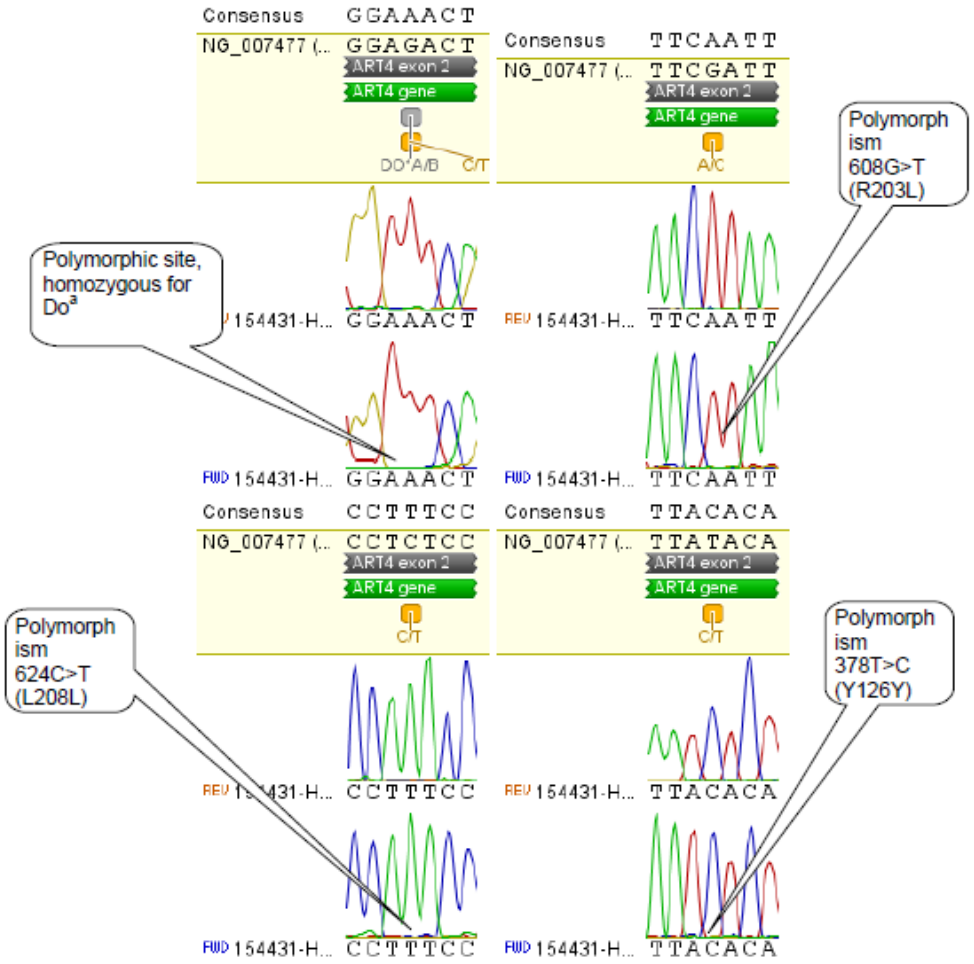
Genotyping Results



Sequence study:

The molecular presence of Adenine at the polymorphic site that is indicative of the Do^a antigen represents a homozygous Do^a individual.

The sample is also homozygous for a missense mutation at 608G>T (R203L). This polymorphism has no known clinical impact.

The sample is also homozygous for two silent mutations at 624C>T (L208L) and 378T>C (Y126Y).

No other polymorphisms were found in the coding region of *ART4,* the gene encoding the DO system

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DO system characteristics

- The Dombrock (DO) system consists of seven antigens and Gy^a is a high prevalence antigen in the DO system

- DO antigens are: resistant to ficin and papain treatment, weakened with a-chymotrypsin and sensitive to DDT200mM

- DO antibodies are usually IgG, predominantly IgG1

- Anti-Gy^a is the antibody characteristically produced by immunized individuals with the Dombrock-null phenotype, which results from various inactivating mutations

- Anti-Gy^a has been reported to cause transfusion reactions but has not been implicated in HDFN, although a positive DAT in the newborn has been detected

- Siblings of patients with anti-Gy^a should be tested for compatibility and the patient urged to donate blood for cryogenic storage when clinical state permits

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Updated Clinical Information

- The patient delivered at the 40th week of gestation
- The newborn presented no clinical symptoms of HDN
- No units were available in our inventory and only 4 possible matches were detected at International Rare Donor Panel
- No autologous units were available
- No transfusion support was required by either mother or child

Conclusions

- The role of IRL in identifying rare antibodies and in finding negative blood for a rare phenotype is very relevant

- In these cases programs of predepositing and freezing of the immunized subject's red cells and the typing of relatives are recommended

- An adequate counselling and the availability in National/International Banks or Registers of frozen rare phenotype units help to provide a safe transfusion therapy

Lessons Learned by the Case

- Although DNA typing for the prediction of blood groups has great value, there are several limitations

- There are many genetic events that cause apparent discrepant results between hemagglutination and DNA typing

- The genotype is not the phenotype

- Confirmation of predicted phenotype is recommended using different technologies such as serology

- In our case report, a false positive genotyping results would have not allowed a correct antibody identification

References

- Geoff Daniels. Human Blood Groups. Third Edition Wiley-Blackwell

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