Immunohematology Case Studies 2018 - 6

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Clinical History

Medical history:

A 61 years old female, alcoholic with liver failure and esophageal varices urgently operated for intestinal obstruction.

Transfusion history:

- June 2003: 2 red blood cell units transfused
- July and September 2005: 3 red blood cell units transfused in this time period

Pregnancy history: unknown

Serologic History

- Allo-anti-E and allo-anti-K identified in 2005
- Routine serological workup was performed
- The referring hospital transfusion service observed that antibody screen and identification were positive with all cells, DAT negative
- Due to limited resources, a sample was submitted to Immunohematology Reference Laboratory at Policlinico Hospital of Milan (Italy) for further testing

Current Sample Presentation Data

ABO/Rh/K: O Rh positive, CCee, kk

DAT: negative

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

Antibody Screen Results: 3+ with all tested cells

Antibody Identification Method: IAT using CAT-Polyspecific, polyethylene glycol (PEG), and ficin-treated cells

Antibody Identification Preliminary Results: all cells positive in IAT with untreated and ficin-treated red cells

Antibody Identification
Preliminary Results

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

AC: autocontrol

Challenge with the Current Presentation

- Pattern of pan-agglutination observed

- The most frequent causes for the “all cells positive/DAT negative” pattern are:
    - Multiple antibodies to common antigens
    - An antibody to an antigen of high prevalence
    - An antibody to reagent components
    - Presence of administered therapeutic monoclonal antibodies eg. anti-CD38

- The laboratory performed further tests

Further Serologic Work

The extended phenotype for common red blood cell antigens and other rare high frequency antigens (HFA) was investigated, with the following results:

Antisera	Patient RBCs	Antisera	Patient RBCs	Antisera	Patient RBCs
C^w	0	s	+	U	+
K	0	Kp^b	+	Co^a	+
k	+	Js^b	+	Vel	+
Jk^a	+	Lu^b	+		
Jk^b	+	PP_1P^k	+		
Fy^a	0	Gy^a	+		
Fy^b	+	Lan	+		
S	0	Ge2	+		

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**	**Kp^b**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**	**S**	**s**	**CAT**	**S20**	**AHGT IgG**	**Ficin IgG**	**DTT IgG**	**Trypsin IgG**
I042818010596	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	2+	0	1+	2+	2+	2+

- 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
- The antibody was reactive with chemically-modified and enzyme-modified red cells

Supplementary Tests: Adsorption

- Antibodies to high-prevalence antigens may be accompanied by antibodies to common antigens, which can make identification much more difficult
- 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
- This approch leaves antibodies to common red cell antigens in the adsorbed plasma, where they can be identified with a routine selected red cell panel
- The adsorption should not solely be with cells of the patient's phenotype because if the patient has a variant allele and has formed an antibody to the wild type antigen, it will be missed if phenotypically matched cells are used for the adsorption.

Phenotype of adsorbing cell: O, CCDee, K-, Fy(a-), S-

	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	+	0
3	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0
4	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0
5	0	0	+	0	+	0	0	+	0	+	+	0	0	+	+	0	+	0	+	2+
6	+	0	+	0	+	0	0	+	0	0	+	+	0	0	+	0	+	0	+	2+
7	+	+	0	0	+	0	+	0	+	+	+	0	+	0	0	+	0	0	+	0
8	+	+	0	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	+	0
9	+	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	+	+	+	0
10	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	0

An allo-anti-c was identified after alloadsorption of the plasma

Supplementary Tests: Eluate

- Combined adsorption-elution test can be used to separate a mixture of antibodies
- Both the eluate and adsorbed serun can be used for futher testing
- Eluate from red blood cell unit (phenotype of adsorbing cell: O, CCDee, K-, Fy(a-), S- was tested:

	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	+	+	+	+	3+
2	+	+	0	0	+	+	0	+	+	0	0	+	0	0	+	+	+	0	+	3+
3	+	0	+	+	0	0	0	+	0	+	+	0	+	0	+	+	+	+	+	3+
4	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	+	3+
5	0	+	+	0	+	0	0	+	+	+	+	0	0	+	+	0	+	0	+	0
6	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	0	+	0	+	0	+	0	0	+	0
9	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0
10	+	w	+	+	0	0	0	+	0	0	+	0	0	+	+	+	+	+	0	3+
11	+	0	+	0	+	0	0	+	0	0	+	0	0	+	+	0	+	0	0	3+

An allo-anti-D was identified in the eluate obtained after alloadsorption on R_1R_1 cells

Supplementary Tests: serology vs molecular

Informazioni sul campione — **ID paziente: -**

ID campione: **136699**

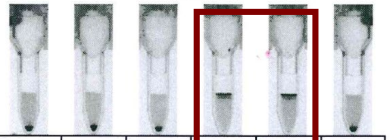
Posizione: **Rotore campione 1Rack 3 Pos 4**
Tipo di liquido: **CENTBLOOD**
Priorità: **Routine**

ABO(FWD)-ABODD-48 — ABO: **O** Rh: **POS**

Ora avvio: 03/05/2018 13:40 - Ora completamento: 03/05/2018 13:50

Risultati

Cassetta 1



Risultato	Anti-A	Anti-B	Anti-A+B	Anti-D	Anti-D	Ctrl
Originale	0	0	0	4+	4+	0
Modificato						

ID cassetta: 101018-48-023102-27610-7 Lotto: 27610 Scad. 10/10/2018

Indicatori:
Accettato da: Sistema

Patient's type for RhD by serology and molecular biology

BioArraySolutions

wRHD BeadChip™ Chip Assignment Report

Printed: 5/17/2018 11:11:40 am

Chipname	SampleName	Notes/Status	WarnMsg	Variant
RHDAN849_1	2018-063-136699		LS(1)	DNB (hemizygous or homozygous)

Patient has a DNB phenotype

DNB phenotype

DNB: a partial D with anti-D frequent in Central Europe

Franz F. Wagner, Nicole I. Eicher, Jan R. Jørgensen, Cornelie B. Lonicer, and Willy A. Flegel

To improve routine D typing and define transfusion strategy, it is important to establish the frequency of partial D alleles and their susceptibility to anti-D alloimmunization due to transfusion or pregnancy. We identified the partial D DNB that was caused by an *RHD*(G355S) allele associated with a *CDe* haplotype and whose phenotype presented a normal D in routine typing. The antigen density was about 6000 D antigens per red blood cell, and the Rhesus index was 0.02. Five anti-D immunization events with allo–anti-D titers up to 128 were observed. Twelve carriers of DNB were whites of Central Europe; the only Danish proband had Austrian ancestry. DNB was the most frequent partial D recognized so far in whites, occurring with frequencies of up to 1:292 in Switzerland. DNB was the underlying partial D phenotype in a relevant fraction of anti-D immunizations occurring in whites. (Blood. 2002;100: 2253-2256)

BLOOD, 15 SEPTEMBER 2002 • VOLUME 100, NUMBER 6

Common category or name Phenotype(s)	Allele name	Nucleotide change	Exon(s)	Amino Acid(s)	Allele Name Detail
DNB	***RHD*25*** ***RHD*DNB***	c.1063G>A	7	p.Gly355Ser	*RHD*1063A*

http://www.isbtweb.org/fileadmin/user_upload/Working_parties/WP_on_Red_Cell_Immunogenetics_and/RHD_Partial_D_blood_group_alleles_v5.0_180207.pdf

DNB phenotype

- DNB phenotype has a normal antigen density and is agglutinated by most anti-Ds, including almost all commercial anti-D typing reagents

- A serologic characterisation of DNB is difficult

- The anti-D immunization risk in DNB carriers may safely be estimated to be lower than 1% per D-positive transfusion. This immunization index was less than known for anti-K and anti-c but may be comparable to that of anti-Fy^a and anti-Jk^a.

- A recipient to carry a DNB phenotype should be transfused with D-negative red cells

Updated Clinical Information

- Due the presence of anti-D and anti-c, the only units suitable for transfusion have a rare phenotype CCdee

	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-AS
ID 040511101624	0	+	0	0	+	/	0	+	0	+	+	+	/	/	/	/	/	0	+	0
Cell 5 Panel 10	0	+	0	0	+	0	0	+	+	+	+	0	0	+	+	0	+	0	+	0

- No autologous units were available
- No fresh units were available but were present in our frozen inventory
- No transfusion support was required

Conclusions

- Within the RH blood group system, the D (RH1) antigen is the most immunogenic and of great clinical significance due to its direct implication in hemolytic transfusion reactions and in hemolytic disease of the fetus and newborn

- Many variants of the RhD protein have been described molecularly to result from one or more amino acid substitutions or *RHD-CE-D* gene conversion events

- Some partial D go unrecognized in testing at immediate spin because the RBCs type strongly D+ in direct test

- Anti-D immunizations may occur in “partial D” carriers after exposure to conventional D antigen via blood transfusion or pregnancy

Lessons Learned by the Case

- Perform RHD genotyping on patients D+ with anti-D antibodies
- Partial D status is a major concern for transfusion and pregnancy, due to the possibility of carriers becoming immunized
- More than 500 *RHD* alleles have been reported
- Alterated D is organized into four groups:
    - weak D: red cells with a reduced amount of D antigen that required an indirect antiglobulin test (IAT) for detection due to missense mutations in regions of *RHD* encoding transmembrane/cytoplasmic portion of D**.** Type 1,2,3 represent approximately 90% of the weak D types in persons of European ethnicity.
    - partial D: red cells type as D positive but lack exofacial epitopes due to hybrid proteins and missense mutations affecting exofacial protein
    - D_{el} types: red cells that express extremely low levels of D antigen that can be detected by adsorption/elution studies
    - nonfunctional *RHD: RHD* genes that do not encode a full-length functional polypeptide

References

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