Immunohematology Case Studies 2020 - #5

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Clinical History

- 21 year old Caucasian female at 40 weeks gestational age
- G2P1
- No history of blood transfusion

Serologic History

- The referring laboratory tested multiple panels
- Most cells were positive on these preliminary panels
- The referring facility suspected a possible anti-Jk^b with anti-E

Current Sample Presentation Data

ABO/Rh: B/D-positive

DAT: IgG- negative, C3b/d- negative

Antibody Screen Method: PeG-IAT and LISS-IAT (Immucor enhancements, Alba Screen Cells)

Phenotype:

D+ C+ E– c+ e+, K–, Fy(a–b+), Jk(a+b+), M+ N– S+ s+

Preliminary Testing:

Phenotype: D+C+E–c+e+; K–; Fy(a–b+); Jk(a+b+); M+N–S+s+

Antibody Screen: PeG-IAT and LISS-IAT

			Rh-hr								Kell						Duffy		Kidd		Lewis		MNS				P	Lutheran		Additional Antigens			TEST RESULTS			
Cell #	Rh-hr	Donor	D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b	Xg^a	Wr^a	Special Types				
1	$R_1^wR_1$	2768020059071	+	+	0	0	+	0	NT	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	+	0				
2	R_2R_2	6603030518015	+	0	+	+	0	0	NT	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	0	0	+	+	0				
3	rr	2768020217271	0	0	0	+	+	+	NT	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	+	0	0	0	+	+	0				
Patient Cells																																			

Selected Cell Panel based upon phenotype:

	Supplier / Lot	Donor / RhHr - Vial	Rh-Hr								Kell						Duffy		Kidd		Lewis		P	MN				Luth		X	Additional Antigens		
			D	C	E	c	e	C^w	f	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a				
1	Medion 612018017	M4215CC rr #5	0	0	0	+	+	0	+		0	+	+		0		0	+	0	+	0	+	+	+	0	0	+	0	+	+	phenomatched			
2	Immucor 32438	G1834 rr #17	0	0	0	+	+	0		0	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	+	0	+	+				
3	Quotient V199497	2768020335325 R1wR1 #1	+	+	0	0	+	+	0		0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	+	+	0	+	+	Wr(a-)			
4	Immucor 33441	N2775 rr #14	0	0	0	+	+	0		0	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	0	+	0	+	+				
5	Immucor 34457	C6010 R2R2 #3	+	0	+	+	0	0		0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	0	+	0				
6	Ortho-Clinical VRB249	302221 RzR1 #20	+	+	+	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	0	+	0	HLA+			

Suspected anti–E, –K, and –Fy^a (demonstrating dosage, because screen cell #1 was negative and it poses only a single dose of the Fy^a antigen)

Challenge with the Current Presentation

An anti-E, –K, and –Fy^a (demonstrating dosage) were suspected

Question asked of the case:

Does the history of this patient make sense that she would have an anti-E, –K, and –Fy^a?

- She has never been transfused
- Second pregnancy, no complications

Answer: No, that doesn't make sense:

Additional Testing required

Further Work

Cold Panel: Negative

(The cold panel consisted of testing the screen cells, auto control, two cord cells, and two B cells at Saline-IS, 15 minutes Room Temperature, and 15 minutes 4° C)

Gel-IAT panel (Phenomatched cells tested at Ficin-Gel-IAT)

			Rh-hr								KELL						DUFFY		KIDD			LEWIS		MNS				P	LUTHERAN		Special Antigen Typing	Test Results (Ficin)	
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell #	MTS Gel
1	R1wR1	318803	+	+	0	0	+	0	+	0	0	+	0	+	/	+	+	+	+	+	0	0	+	0	+	0	0	+	0	+		1	2+
2	R1R1	319355	+	+	0	0	+	0	0	0	+	+	0	+	/	+	+	+	+	0	+	0	+	0	+	0	+	+	0	+		2	0
3	R2R2	321916	+	0	+	+	0	0	0	0	0	+	0	+	/	+	0	+	+	0	+	0	+	0	+	+	0	+s	0	+	HLA+	3	2+
4	Ror	312177	+	0	0	+	+	+	0	0	0	+	0	+	/	+	0	0	+	0	+	0	0	0	+	+	0	+	0	+	P.M.	4	0
5	r'r	319285	0	+	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	+	+	0	+	+	+	+	0	+s	+	+	@ P.M. close	5	0
6	r"r	321896	0	0	+	+	+	+	0	0	0	+	0	+	/	+	+	0	+	+	+	0	+	+	+	+	0	+	0	+	@	6	2+
7	rr	321863	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	+s	0	+	@, HLA+	7	2+
8	rr	313174	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	@	8	2+
9	rr	305755	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	0	+	+	+		9	2+
10	rr	313370	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	0	+	+	0	0	+	0	+	0	+	+		10	0
11	R1R1	317651	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	0	+	+	0	0	+	0	+	0	0	0	+		11	2+
	Patient Cells																													A/C		0
Mode of Reactivity			37°C/Antiglobulin								Antiglobulin											Variable		Cold			Var.					

Ortho – ID Microtyping Systems- MTS Gel

Interim Antibody Identification
Possible Answers and Next Steps

- Negative cold panel and negative reactions at Ficin-Gel-IAT: autoantibodies not likely cause of reactions observed

- Gel-IAT panel excludes anti–Fy^a with two double dose cells, and anti–K with one single dose cell

Theory: A single antibody is present and causing these reactions.

What antibodies can cause this type of pattern?

Further Work

- All cells tested by the referring laboratory and the IRL were counted to obtain the prevalence of antigen positive cells

  (Duplicate donor cells were not counted)

Serum Reactions	Referring Lab	IRL	Total
Positive	15	6	21
Negative	6	3	9
		Total Tested:	30
		Percent Postive:	70%

Approximately 70% of cells tested are presumed to be positive for the antigen characterized by this antibody.

Refer to the books: One antigen fits this picture

Further Work

- Do^a is present in approximately 67% of Caucasians
- Utilizing the fact that the Do^a antigen is sensitive to 0.2M DTT (dithiothreitol) treatment, the initial antibody screen was performed with 0.2M DTT treated cells, along with a 2 cell screen

			Rh-hr								Kell						Duffy		Kidd		Lewis		MNS				P	Lutheran		Additional Antigens				TEST RESULTS				
Cell #	Rh-hr	Donor	D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b	Xg^a	Wr^a	Special Types			LISS	37	AHG	AHG	Cell #
1	$R_1^wR_1$	2768020059071	+	+	0	0	+	0	NT	+	0	+	0	+	0	+	+	+	0	+	0	+	0	~~+~~	+	+	+	0	+	+	0				0	0	0	0	1
2	R_2R_2	6603030518015	+	0	+	+	0	0	NT	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	0	+	+	0				0	0	w+	1+	2
3	rr	2768020217271	0	0	0	+	+	+	NT	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	+	0	0	0	+	+	0				0	0	1+	1+	3
Patient Cells																																						

			Rh-hr								Kell						Duffy		Kidd		Lewis		MNS				P	Lutheran		Additional Antigens			TEST RESULTS				
Cell #	Rh-hr	Donor	D	C	~~E~~	c	e	f	^wV	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	~~Fy^a~~	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b	Xg^a	Wr^a	Special Types						Cell #
1	R_1R_1	1000110042	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	0	0						1
2	R_2R_2	1000249398	+	0	~~+~~	+	0	0	0	0	0	+	0	+	0	+	~~+~~	0	0	+	+	0	0	+	0	+	+	0	+	0	0						2

Further Work

Do(a–) and Do(a+) panels were selected and tested in parallel

Do(a–) panel Results:

	Supplier / Lot	Donor / RhHr - Vial	Rh-Hr								Kell						Duffy		Kidd		Lewis		P	MN				Luth		X	Additional Antigens	MNS Lab #
			D	C	~~E~~	c	e	C^w	f	V	~~K~~	k	Kp^a	Kp^b	Js^a	Js^b	~~Fy^a~~	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	~~N~~	S	s	Lu^a	Lu^b	Xg^a		
1	Immucor 34457	G1376 rr #7	0	0	0	+	+	0		0	~~+~~	+	0	+	0	+	0	+	+	+	+	0	+	0	~~+~~	0	+	0	+	+	* Do(a-)	0
2	Immucor 34457	N3072 rr #9	0	0	0	+	+	0		0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	0	0	+	+	Lu:14 * Do(a-)	0
3	Immucor 34457	B2849 R1R1 #TC	+	+	0	0	+	0		0	0	+	0	+	0	+	~~+~~	0	+	+	+	0	+	0	~~+~~	0	+	0	+	+	Wr(a+) * Do(a-)	0
4	Immucor 30412	A1408 RzR1 #1	+	+	~~+~~	0	+	0		0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	Do(a-), Do(b+) *	0
5	Immucor 30412	D1962 Ror #4	+	0	0	+	+	0		+	0	+	0	+	+	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	Do(a-), Do(b+); * Hy-, Jo(a-), VS+	0
6	Immucor 35462	A4554 R1R2 #11	+	+	~~+~~	+	+	0		0	0	+	0	+	0	+	~~+~~	0	0	+	0	+	0	+	0	0	+	0	+	+	Mi(a+), Mur+ Do(a-)	0

* Indicates cells which the Dombrock type is predicted from genotyping performed by Immucor

The originally suspected anti–E, –K, and –Fy^a have been excluded with Do(a–) cells.

Anti–K has now been excluded with two single dose cells.
(slide 7 and this panel), permitted by laboratory procedure

Further Work

Do(a+) panel Results:

	Supplier / Lot	Donor / RhHr - Vial	Rh-Hr								Kell						Duffy		Kidd		Lewis		P	MN				Luth		X	Additional Antigens	IAT
			D	C	E	c	e	C^w	f	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a		
1	Immucor 30412	G1332 rr #7	0	0	0	+	+	0		0	+	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	0	+	+	Do(a+) Do(b-) *	2+
2	Immucor 30412	T34 rGr #TC	0	w	0	+	+	0		0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	0	Do(a+) Do (b-) *	2+
3	Immucor 29395	A3355 RzR1 #1	+	+	+	0	+	0		0	0	+	+	+	0	+	+	+	0	+	+	0	0	0	+	+	+	0	+	+	Do(a+) Do (b-)	2+
4	Immucor 32438	D2056 Ror #20	+	0	0	+	+	0		+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	0	+	+	VS+ * Do(a+) Do(b+)	it

	Supplier / Lot	Donor / RhHr - Vial	Rh-Hr								Kell						Duffy		Kidd		Lewis		P	MN				Luth		X	Additional Antigens	IAT
			D	C	E	c	e	C^w	f	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a		
1	Immucor 32438	B9642 R1R1 #4	+	+	0	0	+	0		0	0	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	* Do(a+), Do(b+)	2+
2	Immucor 32438	C4587 R2R2 #8	+	0	+	+	0	0		0	0	+	0	+	0	+	+	+	+	0	0	+	0	0	+	+	+	0	+	+	Do(a+)	2+
3	Immucor 31417	A4443 RzR1 #1	+	+	+	0	+	0		0	0	+	0	+	0	+	+	+	+	+	0	+	0	0	+	0	+	0	+	+	* Do(a+) Do(b+)	2+

* Indicates cells which the Dombrock type is predicted from genotyping performed by Immucor

Note: The top panel was tested in parallel with the Do(a–) panel on the previous slide

Genotyping Results

HEA BioArray
(Immucor)

Patient predicted to be
Do(a–b+)

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	+	
	e	+	
	E	0	
	V	0	
	VS	0	
Kell	K	0	
	k	+	
	Kp^a	0	
	Kp^b	+	
	Js^a	0	
	Js^b	+	
Duffy	Fy^a	0	
	Fy^b	+	
Kidd	Jk^a	+	
	Jk^b	+	
MNS	M	+	
	N	0	
	S	+	
	s	+	
	U	+	
Lutheran	Lu^a	0	
	Lu^b	+	
Diego	Di^a	+	
	Di^b	+	
Colton	Co^a	+	
	Co^b	0	
Dombrock	Do^a	0	
	Do^b	+	
	Hy	+	
	Jo^a	+	
Landsteiner-Wiener	LW^a	+	
	LW^b	0	
Scianna	Sc1	+	
	Sc2	0	

Blood Group	Polymorphism	Result
Rh	307C>T (RhCE-P103S)	Ax
	109-bp ins (RhCE-109ins)	AB
	676G>C (RhCE-A226P)	AA
	1006G>T (RhCE-G336C)	AA
	733C>G (RhCE-L245V)	AA
Kell	698T>C (K1/K2)	BB
	961T>C (Kp)	BB
	1910C>T (Js)	BB
Duffy	125G>A (FYA/FYB)	BB
	-67T>C (GATA)	AA
	265C>T (FY-265)	AA
Kidd	838G>A (JKA/JKB)	AB
MNS	59C>T (GPA)	AA
	143T>C (GPBS)	AB
	+5G>T (GPB-Int5)	AA
	230C>T (GPB-230)	AA
Lutheran	230A>G (LUA/LUB)	BB
Diego	2561T>C (DIA/DIB)	AB
Colton	134C>T (COA/COB)	AA
Dombrock	793A>G (DO-793)	BB
	323G>T (DO-323)	AA
	350C>T (DO-350)	AA
Landsteiner-Wiener	308A>G (LWA/LWB)	AA
Scianna	169G>A (SC1/SC2)	AA

Conclusions

- Anti-Do^{a} was identified
- The patient is homozygous for *DO*B*
- The patient's predicted phenotype is Do(a–b+)
- There was no complications with the pregnancy or neonate
- The anti-Do^{a} was likely stimulated as a result of the first pregnancy

Summary of Case Challenges

- Anti-Do^{a} is rarely observed as a monospecific specificity
- Antibody identification panels often do not have Dombrock typings
- The pattern observed in the antibody identification panels could have been misinterpreted as multiple antibodies
- If the antibodies had been misidentified as an anti-K with others, the patient likely would have undergone additional procedures that were unnecessary because of the risk of HDFN due to anti-K

Lessons Learned by the Case

- It is important to reconcile the history of a patient to the test results
- The prevalence of an antigen can be utilized to help guide testing
- Genotyped panel cells are imperative to identify some antibody specificities
- “Stray” positive or negative reactions should not be ignored, they may aid in the identification
- Anti-Do^{a} is usually observed in a mixture of antibodies, but there are a few reported cases of anti-Do^{a} caused by pregnancy

ISBT Terminology of the System

Dombrock Blood Group System

ISBT Symbol: DO (014)

Antigens: 10

Short arm of chromosome 12 (12p12.3)

Gene: *ART4*

Reference Allele*: DO*A*

Exons: 3

Entrez Gene ID: 420

Genomic Sequence: NG_007477.1

Brief Review of the Blood Group System or Antibody

The Dombrock (DO) system is encoded by the *ART4* gene, with 3 exons, which is located on the short arm of chromosome 12. There are 10 antigens recognized by the ISBT. Semi-automated genotyping platforms predict the Do^a and Do^b antigens using the c.793A>G change.

Anti–Do^a and –Do^b can cause delayed hemolytic transfusion reactions yet are notoriously elusive in vitro. Because no licensed antisera exist and polyclonal (human-derived) antisera are not readily available, DNA testing has become the standard for predicting the DO antigens for patients and donors.

References/ Acknowledgement

Working Party on Red Cell Immunogenetics and Blood Group Terminology. Names for DO (ISBT 014) blood group alleles v4.0 160623. Amsterdam: International Society of Blood Transfusion, 2016.

Table of blood group antigens v.9.0_12th July 2019. Amsterdam: International Society of Blood Transfusion, 2019.

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