Case Studies

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Case 1 - A little bit of everything

- Patient LM, 64 y/o female
- Diagnosis: Not provided
- Referred for antibody ID; history of WAA, anti-K, -Fy^a, -Jk^b.
- Transfusion history:
  - 2 units rbcs 3 months prior to referral
  - 2 units rbcs 1 month ago
  - All units group A, Rh negative

Case 1: Initial results

- ABO/Rh

Anti-A	Anti-B	Anti-D	RhC	A_1 cells	B cells
4+	0	0	0	0	4+

- DAT

Polyspecific AHG		Anti-IgG		Anti-C3				Saline
IS	Mi	IS	Mi	IS	Mi	RT	Mi	IS/Mi
1+		1+		0	0	0	0✓	0/0

Antibody screen

	IS	LISS 37	LISS IAT		PeG IAT
SI	2+	4+	4+		4+
SII	2+	4+	3+		4+
SIII	1+	2+	2+		3+
AC	1+	0	1+		2+

Phenotype

- We need autologous cells
  - For phenotype to determine what antibodies this patient can produce
  - To investigate if autoantibody is present in the plasma and eluate
- Patient has been transfused within the last 3 months
  - Pre-trx sample not available

Microhematocrit Cell Separation

- Separation of young autologous red cells (i.e. reticulocytes) from transfused RBCs by simple centrifugation in microhematocrit tubes
- Reticulocytes have a lower specific gravity and therefore are concentrated at the top of the microhematocrit tube
- Best if samples collected 3 or more days after transfusion
- Other method: Hypotonic Saline Wash (used for patients with sickle cell disease)

Phenotyping results

- Cell separation performed
  - Used **R**eticulocyte **E**nriched **P**ortion (REP) for testing

- Phenotype:

  C-E-c+e+; K-; Fy(a-b+); Jk(a+b-); S-s+;

  M+N-; P_1-; Le(a-b-)

- Patient can produce alloantibodies to:
  - D, C, E, K, Fy^a, Jk^b, S, N, P_1, Le^a, Le^b
  - History of previous anti-K, -Fy^a, Jk^b

What to do next?

- Is the autoantibody reported by the referring laboratory currently present in the plasma?
- What is reactive at IS and 37º phases? Could the autoantibody have a broad thermal range, or are these alloantibodies?

Our formula for distinguishing autoantibody from alloantibody

Test DAT negative autologous cells and phenotypically similar cells with patient plasma and/or eluate:

Phen sim pos, auto neg = Probable alloantibody to HIA

Phen sim pos, auto pos = Probable autoantibody

Phen sim neg, auto neg = Probable multiple alloantibodies

Is Warm autoantibody there?

	D	C	E	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s		IS	LISS 37	LISS IAT	PeG IAT
PS	0	0	0	0	0	+	+	0	0	+		0	0	1+	2+
REP	0	0	0	0	0	+	+	0	0	+		0	0	1+	2+
CTL	(REP tested with 6% albumin as control)											0	0	0✓	0✓

Antibody screen

	IS	LISS 37	LISS IAT		PeG IAT
SI	2+	4+	4+		4+
SII	2+	4+	3+		4+
SIII	1+	2+	2+		3+
AC	1+	0	1+		2+

Antibody Identification - Selected Cell Panel

	Rh-Hr					Kell		Duffy		Kidd		Lewis		P	MNSs						
Cell #	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IS	LISS 37	IAT
1	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	+	0	+	2+	3+	4+
2	0	0	0	+	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	0	1+
3	0	+	0	+	+	0	+	0	+	+	0	0	+	+	+	+	0	+	2+	4+	4+
4	0	0	+	+	0	0	+	0	+	+	0	0	+	+	+	+	0	+	1+	2+	2+
5	0	0	0	+	+	0	+	+	0	+	0	0	0	+	0	+	0	+	0	0	2+
6	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	1+
7	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	0	1+
8	0	0	0	+	+	+	0	0	+	+	0	0	+	+	+	+	+	0	1+	1+	3+
9																					
10																					
11																					

Antibody Identification - Selected Cell Panel

	Rh-Hr					Kell		Duffy		Kidd		Lewis		P	MNSs						
Cell #	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IS	LISS 37	IAT
1	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	+	0	+	2+	3+	4+
2	0	0	0	+	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	0	1+
3	0	+	0	+	+	0	+	0	+	+	0	0	+	+	+	+	0	+	2+	4+	4+
4	0	0	+	+	0	0	+	0	+	+	0	0	+	+	+	+	0	+	1+	2+	2+
5	0	0	0	+	+	0	+	+	0	+	0	0	0	+	0	+	0	+	0	0	2+
6	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	1+
7	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	0	1+
8	0	0	0	+	+	+	0	0	+	+	0	0	+	+	+	+	+	0	1+	1+	3+
9																					
10																					
11																					

Summary so far…

- What we have confirmed in the plasma:
  - Warm-reactive autoantibody reactive at IAT phase in PeG and LISS testing
- What we suspect:
  - Anti-D, -C, -E, -K reactive at IS and 37° phases
- What we have left to do:
  - Confirm -D, -C, -E, -K at IS and 37° (need 2 reactive cells for each specificity)
  - Perform allogeneic adsorption to confirm and r/o common alloantibodies at IAT phase.
  - Perform elution; confirm WAA in eluate

Eluate results

- Presence of autoantibody confirmed in the eluate

	Eluate	Last wash
SI	2+	0✓
SII	2+	0✓
SIII	2+	0✓
REP	2+	0✓
P.S.	2+	0✓

Allogeneic adsorption

- Goal is to remove the autoantibody reactivity by adsorption, while leaving any significant alloantibodies in the plasma.
- Adsorbing cells should lack the antigens that the patient can produce antibody to, so the alloantibody(ies) are not adsorbed.
- We chose to adsorb on D-C-E-K-Jk(b-) red cells after papain-treatment (which destroys MNS and Fy antigens)

Antibody Identification - Selected Cell Panel

	Rh-Hr					Kell		Duffy		Kidd		Lewis		P	MNSs				Tests with adsorbed plasma (x1)	
Cell #	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s		PeG IAT
1	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	+	0	+		3+
2	0	0	0	+	+	0	+	0	+	+	0	0	+	+	+	0	0	+		0✓
3	0	+	0	+	+	0	+	0	+	+	0	0	+	+	+	+	0	+		3+
4	0	0	+	+	0	0	+	0	+	+	0	0	+	+	+	+	0	+		2+
5	0	0	0	+	+	0	+	+	0	+	0	0	0	+	0	+	0	+		2+
6	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+		1+
7	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	0		1+w
8	0	0	0	+	+	+	0	0	+	+	0	0	+	+	+	+	+	0		2+
9	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	+	+	0		0✓
10	+	0	0	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+		3+
11	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	0	0	+		3+
12	0	0	0	+	+	0	+	0	+	+	0	+	0	0	0	+	+	0		0✓

Testing done to finish up…

- Additional testing was completed with neat plasma that confirmed presence of anti-D, -C, -E, and -K at IS and 37º phases of testing.
- Additional testing was completed with adsorbed plasma that confirmed the presence of anti-D, -C, -E, -K, -Fy^a, and -Jk^b at the IAT phase of testing.
- The presence of anti-S and anti-N was excluded.

Hmmm, something doesn't make sense….

- The patient was only transfused with Rh negative rbcs
  - Confirmed by referring facility
  - Patient received no platelets or RhIG products
- Where did the anti-D come from???

The G antigen

- G antigen is due to an amino acid that is present on Rh proteins that express the C or D antigen
- The G antigen is rarely expressed on D-C- red cells (r^G and r''^G)
- There are also rare examples of D+ cells that do not express G

Antibody Identification - Selected Cell Panel

	Rh-Hr					Kell		Duffy		Kidd		Lewis		P		Neat plasma	
Cell #	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	Other typings	IS	LISS 37
1	0	0	0	+	+	0	+	0	+	+	+	0	+	0	D-C-, G+	2+	3+
2	0	0	0	+	+	0	+	+	+	+	+	0	+	+	D-C-, G+	2+	3+
3	0	0	0	+	+	0	+	+	0	+	0	0	0	+	(G-)	0	0
4	0	0	0	+	+	0	+	0	+	0	+	0	+	+	(G-)	0	0

- Presence of Anti-G confirmed at IS and 37º phases of reactivity

- The presence of anti-C and -D were not specifically excluded
  - Would require use of adsorption studies
  - Academic interest only, except in cases to evaluate need for RhIG

Conclusions

- Antibodies present in plasma:
  - Anti-G reactive at IS, LISS 37º
  - Anti-K, -E reactive at IS, LISS 37º, PeG-IAT
  - Anti-Fy^a, -Jk^b reactive at PeG-IAT
  - Warm-reactive autoantibody reactive by PeG-IAT and LISS-IAT
  - Not ruled out: Anti-D and -C
- DAT positive (IgG); Eluate: Panagglutinin
- Trx. Recommendation: ABO compatible rbcs negative for D, C, E, K, Fy^a, and Jk^b, crossmatch compatible with neat or adsorbed plasma.

Case 2 - Do you like to gamble?

- Patient MS, 21 y/o male
- Diagnosis: Sickle cell crisis
- Referred for STAT Antibody identification with 4.8 hemoglobin
- Transfusion history
  - 2 units rbcs transfused 8 days prior to sample
  - No other history given

Case 1: Initial results

- ABO/Rh

Anti-A	Anti-B	Anti-D	RhC	A_1 cells	B cells
0	0	4+	0	4+	4+

- DAT

Polyspecific AHG		Anti-IgG		Anti-C3				Saline
IS	Mi	IS	Mi	IS	Mi	RT	Mi	IS/Mi
1+		1+		0	0	1+		0/0

Antibody screen and Eluate

	IS	LISS 37	LISS IAT	PeG IAT		Eluate	Last wash
SI	0	0	0✓	2+		3+	0✓
SII	0	0	0✓	2+		3+	0✓
SIII	0	0	0✓	2+		3+	0✓
AC	0	0	1+	1+		NT	NT

LISS panel

Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		Xg	PATIENT'S SERUM TEST RES TEST METHODS				
D	C	c	E	e	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	P_1	M	N	S	s	Lu^a	Lu^b	Xg^{*a}		LKS 37	46	cc	
+	+	0	0	+	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	+	0	0	+	0	+	0	1	0	0	✓	
+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	2	0	0	✓	
+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	3	0	0	✓	
+	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	0	0	0	+	+	4	0	0	✓	
0	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	+	0	0	0	+	+	+	0	0	+	+	5	0	0	✓	
0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	0	+	+	+	+	0	+	+	+	+	6	0	0	✓	
0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	0	+	+	7	0	0	✓	
0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	0	+	0	+	0	8	0	0	✓	
0	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	+	0	0	+	+	9	0	0	✓	
+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	+	10	0	0	✓	
+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	+	0	0	+	+	0	+	0	0	+	+	TC	0	0	✓	

Can we interpret these results?

- Only one small EDTA sample was sent from the referring hospital; only enough plasma left for crossmatching.
- No red cells remaining for cell separation and phenotype (used to prepare eluate).
- This looks like a warm autoantibody; alloantibodies are ruled out by LISS testing. Should we send units because this is a STAT, or ask for more sample to complete the investigation?

More work to do…

- Received additional sample
- Need autologous cells and phenotype
  - Patient recently transfused
- Confirm presence of autoantibody

Phen sim pos, auto neg = Probable alloantibody to HIA

Phen sim pos, auto pos = Probable autoantibody

Phen sim neg, auto neg = Probable multiple alloantibodies

Phenotyping results

- Cell separation performed
  - Used **Hypotonic Saline Wash method**
  - 0.3% saline lyses normal donor rbcs but leaves HbSS cells intact

- Phenotype:

  C-E-c+e+; K-; Fy(a+); Jk(b+); S-s-; U-

  Fy^b and Jk^a typings inconclusive (weaker than expected reactions)

- Patient can produce alloantibodies to:
  - C, E, K, [Fy^b, Jk^a], S/s/U

Is Warm autoantibody there?

	D	C	E	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s		IS	PeG IAT	Eluate
U+	+	0	0	0	0	0	0	+	0	+		0	2+	3+
U-PS	+	0	0	0	0	0	0	+	0	0		0	0✓	0✓
REP	+	0	0	0	0	?	?	+	0	0		0	0✓	0✓
CTL	(REP tested with 6% albumin as control)											0	0✓	0✓

Summary

- Additional U- cells were tested with plasma and eluate to confirm anti-U and rule out other common allos by PeG-IAT.
- A delayed transfusion reaction (with antibody to HIA or to multiple antigens) can easily be confused serologically and clinically with WAIHA
  - Positive DAT; may appear mixed-field, but MF is hard to detect if the reactivity is weak
  - All cells reactive with plasma and eluate, including the AC if the patient's rbc sample includes donor rbcs
  - Signs of hemolysis - may include unexplained drop in Hct/Hgb, jaundice, hemoglobinuria, etc.
- Transfusion history is important!

Happy Spring!