Tools and Strategies Useful for Antibody Identification

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1st case: 85 y.o. white female

- Admitted for a total knee replacement
- Hgb/Hct: 7.7 gm/dL; 21.3 %
- No record at the referring facility
- Antibody Screen: by gel

	IAT
I:	3+
II:	0

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Initial IRL results

- Gr. O, Rh Negative
- DAT: Weak positive (IgG: Neg; C3: weak pos.)
- Antibody Screens

	I.S.	PeG-IAT	R.T.	37C	IAT
I	W+	2+	1+	0	2+
II	W+	2+	1+	0	2+
III	0	1+	0	1+	1+
AC	0	0 √	0	0	0√

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Phenotyping Patient RBCs

Monoclonals:	C	E	c	e	K
	0	4+	4+	4+	w+/MF

IAT:	Fya	Fyb	Jka	Jkb	S	s
	2+	2+	0	2+	w+/MF	2+

Mixed field reactions in K and S typings indicate recent transfusions with red cell products.

Cell separation (microhematocrit): DAT: 0, K: 0, S: 0

Additional patient history….

- Anti-D identified in 2002
- Patient received 2 RBCs 11 days prior to collection of this sample

- Rethink the initial findings
  - DAT : C3 coating----could this be due to a delayed transfusion reaction?
  - Eluate: Negative

Initial selected cells

	D	C	E	c	e	K	Fy a	Fy b	Jk a	Jk b	S	s		IS	37C	IAT
1	0	0	0	+	+	0	0	+	0	+	0	+		0	0	0v
2	0	0	+	+	+	+	+	+	0	+	0	+		0	W+	W+
3	0	+	0	+	+	0	0	+	0	+	0	+		0	0	0v
4	0	0	0	+	+	0	0	+	+	+	0	+		1+	0	2+
5	0	+	0	+	+	0	+	0	0	+	+	+		0	0	0v

Additional selected cells

	D	C	E	c	e	K	Fy a	Fy b	Jk a	Jk b	S	s		IS	37C	IAT
1	+	0	+	+	0	0	0	+	0	+	+	0		0	0	0v
2	0	0	+	+	+	++	+	+	0	+	0	+		0	1+	1+
3	0	+	0	0	+	+	0	+	0	+	0	+		0	W+	W+
4	0	0	0	+	+	0	0	+	+	+	0	+		1+	0	2+
5	0	0	0	+	+	0	0	+	+	0	+	+		1+	0	2+
6	+	0	0	+	+	0	+	0	0	+	+	+		0	0	0v

Conclusions: 1st case

- *Anti-K* weakly reactive at 37C and at IAT
- *Anti-Jk^{a}* reactive at I.S. and at IAT; IgM component—primary response?
- Previously identified *anti-D* not detected
- Common antibodies to other RBC antigens were ruled out
- Weak Positive DAT; eluate non-reactive
- Monitor patient for further drop in Hgb.

Case #1: Tools and strategies

- Additional patient history; previous anti-D
- Additional test phases helped to separate specificities and rule out other IgM antibody
  - I.S. and R. T.; 37C
- Red cell phenotyping and cell separation
  - Revealed that pt. had been recently transfused
  - Identified which alloantibodies pt. can make
- Eluate: Helped to rule out add'l antibodies

2nd Case: 30 y.o. Caucasian female

- Scheduled for C-section the next day
- G: 2; P-1
- No BB record at this hospital
- Meds: vitamins, Aldomet, tylenol
- Antibody screen: Gel

	IgG
I	1+
II	0
III	2+

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Initial IRL findings

- Gr. O, Rh Positive
- Solid phase testing:
  - DAT: invalid
  - Antibody screen: 4+ with 4/4 cells
- Manual testing:
  - DAT; 2+; IgG: 2+; C3: Neg.
  - Antibody screen in gel: 1+ with 3/3 cells

Phenotyping Patient RBCs

- Monoclonals:

C	E	c	e	K
+	0	0	+	0

- EGA (EDTA-glycine) treatment to remove IgG
  - Repeat DAT: *Negative*

  - IAT:

6% alb	Fya	Fyb	Jka	Jkb	S	s
0√	+	+	+	0√	0√	+

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Additional testing with DAT negative patient RBCs

- ***Plasma:***
  - Auto control (EGA-ttd) in gel: 2+ (6% albumin control: 0)
  - Phenotypically similar cells: 2+

- ***Eluate:***
  - Auto control (EGA-ttd) in gel: 2+ (6% albumin control: 0)
  - Phenotypically similar cells: 2+

Interpretation: Autoantibody (WAA ?) is present in plasma and in the eluate

Are alloantibodies present?

- Options to rule out or detect alloantibodies
  - Absorptions: Auto or allogeneic using pheno-matched RBC
  - Test plasma by less sensitive method (LISS or saline)

- Antibody screen repeated in LISS: Negative

Interpretation: No alloantibodies detected

Return to patient history

Drug: Aldomet (methyldopa): Reported to induce apparent autoantibody that is serologically indistinguishable from WAA

- Reacts *in vitro* without the presence of drug

- Increased frequency of patients on this drug referred to our lab, including several pregnant women

  - *Is there increased usage of the drug, especially for gestational-induced hypertension?*

Methyldopa induced autoantibody

- 10-30% of patients taking drug develop autoantibodies within 3-6 months of therapy
  - Incidence of positive DAT is dose dependent
- 0.5% of patients with a positive DAT have hemolytic anemia
- Removal of drug -- disappearance of autoantibody within 2 weeks but DAT might remain positive for up to 2 years.

From: Petz LD and Garratty G. *Immune Hemolytic Anemias*, 2nd ed. Philadelphia, Churchill Livingstone. 2004.

Autoimmune Hemolytic Anemia (AIHA) in Pregnancy

- AIHA is not uncommon in young women
- Literature reports AIHA occurring 4X more often in pregnant than in non-pregnant females
- Clinically, women respond well to therapy
- Requires close monitoring; corticosteroids or IVIg therapy is generally effective
- Fetus: Minimal or no evidence of hemolysis even when mother has severe anemia

From: Petz LD and Garratty G. *Immune Hemolytic Anemias*, 2nd ed. Phil., Churchill Livingstone. 2004.

Conclusions: 2nd Case

- Autoantibody found on RBCs and in plasma
- No alloantibodies were detected
- Clinical correlation needed to distinguish drug (methyldopa)-induced autoantibody from warm reactive autoantibody due to autoimmune hemolytic anemia.
- *Healthy baby was delivered by C-section*

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Case #2: tools and strategies

- EGA (EDTA-glycine) treatment to dissociate IgG from DAT positive cells →*DAT Negative*
  - To confirm that panagglutinin demonstrated in plasma and in eluate is **auto**antibody
  - To perform RBC phenotyping by IAT
- After confirming autoantibody, less sensitive test method used to avoid detection of autoantibody and to detect or rule out alloantibodies.
  - Less risk than performing absorptions

Case #2: tools and strategies—*cont.*

- **Auto**absorption could have been performed
  - Requires adequate volume of autologous RBCs
  - Risk of missing weak antibodies due to dilution
    - Can use enhancement with absorbed plasma
  - More time consuming
- **Allo**absorption could have been performed
  - Risk of absorbing antibody to a high incidence antigen
  - Requires availability of pheno-matched RBCs
  - Risk of missing weak antibodies due to dilution
    - Can use enhancement with absorbed plasma
  - More time consuming

Questions?
Comments?

STAY TUNED FOR ACT II