Immunohematology Case Studies 2017 - #12

Sandra Nance, MS, MT(ASCP)SBB
American Red Cross
National Reference Laboratory for Blood
Group Serology (NRLBGS)
University of Pennsylvania
Sandra.Nance@redcross.org

Clinical History

59 year old Caucasian male

Admitted through Emergency Department

Symptoms included:

- Shortness of breath
- Fatigue

No previous history of hospitalization

Patient states no transfusions, ever

Relevant Laboratory Values

Laboratory Parameter	Patient's Value	Normal Range
Hemoglobin	5.4 g/dL	11.1- 15.9 g/dL
Reticulocyte Count	5%	0.5-2.5%
Bilirubin (Total)	2.5 mg/dL	0-1.2 mg/dL

Serologic Testing at Hospital

Automated Gel testing:

    Antibody Screen:

        1+ with all three antibody screening RBCs

    Gel antibody identification panel:

        3 RBCs negative

        8 RBCs positive: wk+ to 1+

        Autocontrol: negative

    2nd Gel antibody identification panel:

        2 RBCs negative

        9 RBCs positive: wk+ to 1+

        Autocontrol: negative

Current Sample Data from Hospital Testing

ABO/Rh: A positive

DAT: negative with anti-IgG in Gel DAT

Antibody Screen Method: Ortho Gel Test

    Antibody Screen Results: Positive

Antibody Identification Method: Ortho Gel Test

    Antibody Identification initial testing:

        No specificity identified by reactivity

Challenge with the Current Sample Presentation

The hospital has automated Gel testing only, and performs antibody screen and has purchased 2 panels for Gel testing only

The hospital has no other identification methods or panels, and refers the sample to their blood center immunohematology testing laboratory (IRL)

The physicians in charge of the patient manage the patient medically, awaiting antibody identification for compatible blood

IRL Initial Testing

ABO/Rh: O+

DAT: negative with polyspecific AHG, anti-IgG, anti-C3 and control

Rh Phenotype: D+ C+ E- c+ e+

noted that C typing 1+ at Immediate Spin and 4+ after incubation

Further Work - IRL

IRL Technologist evaluates the hospital information, no discernable specificity in Gel panels, decides to perform tube testing in PEG with similar sensitivity*

	D	C	c	E	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	P1	Le^a	Le^b	M	N	S	s	PEG
1	+	+	0	0	+	0	+	0	+	+	+	+	+	0	0	+	0	+	+	0	+	0	1+
2	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0	2+
3	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	2+
4	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+	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	+	2+
7	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+	2+
8	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	2+
9	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+	2+
10	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	2+
A/C																							2+

*PEG – Polyethylene Glycol at antiglobulin phase with Anti-IgG

Evaluate First IRL Panel

IRL Technologist performed tube test to determine the reactivity in other methods

- To establish that testing can be in PEG and Gel testing with specially prepared panel RBCs not needed for evaluation

- PEG panel showed slightly stronger reactivity (2+)

- One panel cell slightly weaker at 1+

- **Autocontrol positive in PEG - 2+ at AHG**

  – Shows importance of performing autocontrol with each method tested

Further Work – Notable Points

#1 RBC was 1+ vs rest of panel 2+, may or may not be significant as was not a 2+ difference

Autocontrol 2+, and DAT was negative!

	D	C	c	E	e	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Jkᵃ	Jkᵇ	Fyᵃ	Fyᵇ	P1	Leᵃ	Leᵇ	M	N	S	s	PEG
1	+	+	0	0	+	0	+	0	+	+	+	+	+	0	0	+	0	+	+	0	+	0	1+
2	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0	2+
3	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	2+
4	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+	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	+	2+
7	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+	2+
8	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	2+
9	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+	2+
10	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	2+
A/C																							2+

*PEG – Polyethylene Glycol at antiglobulin phase with Anti-IgG

Is this Antibody to <u>High Prevalence Antigen</u> or <u>Autoantibody</u>?
Further Testing:

- Sent sample for molecular HEA panel for predicted phenotype of common and some high prevalence antigens
- What if it is an antibody to High Prevalence antigen and DAT result is correct?
  - Allogeneic adsorption - to rule out underlying antibodies to common antigens which could complicate interpretation of testing with rare reagent RBCs, if present

Allogeneic Adsorption using Papain Treated Adsorbing RBCs

#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	R1 Ads PEG IgG	R2 Ads PEG IgG	rr Ads PEG IgG
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√
3	+	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√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0√	0√	0√
AC																								0√	0√	0√

Adsorbing RBCs:
R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr   D- C- E- c+ e+ K-

Note: In NRLBGS, selection of adsorbing RBCs includes S and s typed RBCs to eliminate any concerns with ambiguity of enzyme treatment to eliminate reactivity to S and s antigens

International Society of Blood Transfusion

Allogeneic Adsorption-
No antibodies to common antigens!

#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	R1 Ads PEG IgG	R2 Ads PEG IgG	rr Ads PEG IgG
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√
3	+	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√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0√	0√	0√
AC																								0√	0√	0√

Adsorbing RBCs:
R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr   D- C- E- c+ e+ K-

Is this Antibody to <u>High Prevalence Antigen</u> or <u>Autoantibody</u>? Further Testing:

Allogeneic adsorption – no antibodies to common antigens detected in adsorbed sera - good, now we can proceed

- Subsequent testing considered:
    - Patient's serum with chemically modified RBCs to assist in determining antibody specificity
    - Patient's serum with RBCs negative for high prevalence antigens
    - Patient's RBCs with antisera to high prevalence antigens

Further Work – Test Other Methods to help in Identification of Antibody

Patient's Serum +			
	Antibody Screen Cell #1	Antibody Screen Cell #2	Autocontrol
Ficin Treated RBCs	$+^W$	$+^W$	$+^W$
DTT Treated	$0\sqrt{}$	$0\sqrt{}$	$0\sqrt{}$
Albumin	$0\sqrt{}$	$0\sqrt{}$	$0\sqrt{}$
Gel	1+	1+	1+

Notable results:

1. IRL Gel tests positive, including autocontrol, different from hospital testing (RBCs for Gel test manually prepared) which uses a different solution
2. DTT Treated RBCs non-reactive, ficin weaker, - not typical for autoantibody
    Most likely Blood System implicated: KEL
    Other antigens DTT sensitive and Ficin and Trypsin Resistant: LW
    Variable reactivity antigen to consider: Cr
3. Albumin testing also negative, due to technique or strength of antibody?

Further Antibody Investigation

High prevalence antigen negative RBCs tested:

RBC	Phenotype	PEG AHG Result
1	k-	2+
2	Kp(b-)	2+
3	Js(b-)	0√
4	Lu(a-b-)	2+
5	Yt(a-)	2+

Js(b-) RBCs negative!!!

Further Work – Genotype Results are in

Patient's genotype is in!

Predicted RBC Phenotype is:

D+ C+ c+ E- e+, Fy(a-b+), Jk(a+b+), M+ N+ S- s+ U+, K- k+ Kp(a-b+) Js(a-b+), Do(a+b-) Hy+, Sc:1.-2, Di(a-b+), LW(a+b-), Co(a+b-), Lu(a-b+), Yt(a+b-)

No negative results for high prevalence antigens, and in particular, <u>predicted to be Js(b+)!</u>

Updated Clinical Information

Hospital confirmed:

- Patient was not ever transfused
- Patient is Caucasian

Is this Autoantibody or Antibody to KEL System High Prevalence Antibody?

IRL decided to do autoadsorption first since patient not transfused and autocontrol was positive in some techniques (though DAT negative) rather than thawing rare KEL System high prevalence antigen negative RBCS

Autoadsorption with papain treated patient's RBCs
Tests performed in PEG and read at AHG with anti-IgG, while 3 times adsorbed serum was still positive, four times autoadsorbed patient serum was negative!!!

	D	C	c	E	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	P1	Le^a	Le^b	M	N	S	s	X3	X4
I	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	+^{w}	0√
II	+	0	+	+	0	+	+	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	+^{w}	0√
III	0	0	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	+	+	+	+	+^{w}	0√

Antibody Identification

- Anti-Js^b specificity
- Patient's RBCs typed serologically Js(a-b+)
- No underlying alloantibodies to common antigens detected
- Confounding tests:
  - Negative DAT in tube and Gel tests
  - Positive autocontrol with anti-IgG in:
    - PEG test 2+
    - Ficin test +^{W}
    - IRL Gel test 1+

Testing Interpretations

- Autoadsorption removed anti-Js^b reactivity after four adsorptions
    - IRL considered that the antibody could have been diluted by the 4th adsorption
    - IRL SOP allows up to six adsorptions
    - Therefore, concluded the antibody was adsorbed and was auto-specificity
- Patient's RBCs tested Js(b+)
- Patient's genotype predicted the phenotype as Js(b+)

Testing Not Performed but in Retrospect, of Academic Interest

- Did not test other rare KEL system high prevalence antigen negative RBCs
    - Maybe Js^b antigen is weaker on some rare RBCs and unknown
- Did not perform sequencing to look for variant Js^b
- Did not prepare and test an eluate from the patient's RBCs – even though DAT negative, might have been interesting
- Did not prepare and test and eluate from the adsorbing RBCs – to confirm Js^b specificity

Conclusions

- Patient's report concluded that this antibody was auto anti-Js^b

- Blood needs met by random units the same day

- Patient closely monitored with no laboratory or clinical signs of transfusion reaction

- Patient discharged and has not returned to the hospital

Summary of Case Challenges

Conflicting results between hospital and IRL Gel tests

- Hospital used commercially prepared Gel panels
- IRL prepared 4% panel RBCs with RBC   suspending solution which may have different results than company prepared RBCs

Conflicting results between DAT and autocontrol results

- Untransfused male patient should not have alloantibodies generally thought to be induced by exposure to blood
- Although this initially led IRL staff to test high prevalence antigen negative RBCs, instead of pursuing autoantibody, did lead to anti-Js[b] specificity determination

Lessons Learned by the Case

- The DAT does not always match the autocontrol

- Important to test autocontrol with each different serologic test method, the results could be different

- It helps to assess the serologic reactivity with different test methods

- Always attempt to phenotype the patient for the antigen you are assigning an antibody specificity to, it could be an autoantibody

- Genotyping is essential in complex cases

ISBT Terminology of the KEL System and Brief History

- The ISBT number assigned to KEL is 006 and CD number is CD238

- Js^b is KEL7 (006007) or 6.7

- Obsolete names include Matthews and K7

- Joined the KEL system in 1965 when it was described as being antithetical to Js^a

- Occurrence is 100% in Caucasians and 99% in people of African descent

- As an alloantibody, anti-Js^b has been reported to cause mild to moderate transfusion reaction and mild to severe hemolytic disease of the fetus and newborn

Reid, Lomas-Francis, Olsson. The Blood Group Antigen FactsBook

References

Gordon MC et al. Severe hemolytic disease of the newborn due to anti-Js^b. Vox Sang 1995;69:140-142.

Lee S, Wu X, Reid M, Redman C. Molecular basis of the K:6,-7 [Js(a+b-)] phenotype in the Kell blood group system. Transfusion 1995;35:822–5.

Reid M, Lomas-Francis C, Olsson, M. The Blood Group Antigen FactsBook. 3rd Edition. Elsevier Press 2012.

International Society
of Blood Transfusion

References to possible Auto Anti-Js^b

Eveland D. Autoanti-Js^b enhanced by polyethylene glycol. AABB/ISBT Congress 1990;156 *Abstract*.

Waheed A, Kennedy MS. Delayed hemolytic transfusion reaction caused by anti-Js^b in a Js(a+b+) patient. Transfusion 1982;22:151-162.