Immunohematology Case Studies 2019 – Multiple common antibodies and an antibody to a high-prevalence antigen in a patient with a transplanted bone marrow

Mirela Raos
Head of Transfusion Medicine Division
Clinical Department of Transfusion Medicine and Transplantation Biology
University Clinical Hospital Zagreb, Croatia
mraos@kbc-zagreb.hr

Clinical History

- 41-year-old female Caucasian with Myelodysplastic syndrome (MDS-RAEB-1) was admitted to the hospital for allogeneic unrelated bone marrow transplantation (BMT)
- Two red cell units are immediately ordered (Hgb=60 g/L)

Clinical History

- She had two pregnancies
- During the last delivery she received red cell transfusions
- A month ago she received platelets
- There were no chemotherapeutic regimens before priming

Serologic History

• Indirect antiglobulin test (IAT) was performed on two occasions last year and was negative (the last one was done a month ago when she received platelets)

Current Sample Presentation Data

ABO/D: O D positive

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

Antibody Screen Results: Positive

Antibody Identification Method: IAT using CAT-Polyspecific and Neutral (Biovue, Ortho Clinical Diagnostics) and IAT in tube - IgG

Antibody Identification Preliminary Results: likely anti-E and anti-C^w in IAT with untreated and papain-treated cells, but additional alloantibody is suspected, the autocontrol is negative

Antibody identification panel CAT

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

AC (autocontrol): negative

Antibody identification panel
Tube test

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	RT	37C	IgG
1	+	0	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	2+	2+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	0	0	0
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	0	w
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	0	1+	1+
5	0	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	0	3+	3+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	0	0	1+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	0	0	0
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0	1+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	0	0	w
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0	0	1+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	0	0
AC																				0	0	0

AC (autocontrol) negative
RT = room temperature

Challenge with the Current Presentation

- There are probably anti-E and anti-C^w alloantibodies in the patient's plasma
- Multiple alloantibodies are reactive with all panel cells in IAT with untreated cells and with some enzyme-treated cells, and the autocontrol is negative
- An initial review of results would suggest that an additional alloantibody to a high-prevalence antigen, or more likely multiple alloantibodies, besides anti-E and anti-C^w alloantibodies, are present in the patient's plasma

Interim Antibody Identification
Possible Answers and Next Steps

- Reactivity appears to be an alloantibody to a high frequency antigen, or more likely multiple alloantibodies, besides anti-E and anti-C^w alloantibodies, present in the patient's plasma
- An inconsistency of reactivity with all cells in the panel was shown (the reaction with enzyme-treated cell was not enhanced and with some cells it was negative), and the autocontrol was negative
- Further testing is needed for a conclusion, particularly phenotyping and testing with pheno-matched RBCs

Further Work

- Phenotyping

RBC	ABO	Rh	Kell	Kidd	Lewis	MNS	Duffy	P_1
Patient	O	CcD.ee, C^w neg	K-k+	Jk(a+b-)	Le(a-b+)	M+N+S-s+	Fy(a+b-)	P1+

Updated Clinical Information

- Due to urgency, the patient immediately received 1 O D positive E-, C^w-, K- and Jk(b-), (Fy(b+), S+) red cell unit that was compatible in the tube test, but was positive in CAT (XM in IAT)

  - Pre-transfusion Hgb=60 g/L, DAT negative
  - Post-transfusion Hgb=71 g/L, DAT positive

DAT (CAT): anti-IgG (1+), -IgA, -IgM, C3c, -C3d neg

Eluate: non-specific antibody

Further Work

- Testing with pheno-matched RBCs and papain-treated and 0.2 M dithiothreitol (DTT) treated RBCs, as well as with cord RBCs and autologous RBCs in CAT
- Reaction was positive with 1 unit of pheno-matched O D positive E-, C^w-, K-, Jk(b-), Fy(b-), S- RBCs in IAT (2+)
- After treating the RBCs with papain, the reaction was negative, while after treating RBCs with 0.2 M DTT, it remained positive (1+)
- Antibody reacted in IAT (1+) with cord O D positive E-, C^w- RBCs

Further Testing Results and Interpretations

Adsorption and elution

- RBC phenotypes
  - E+, K-, Jk(b-), Fy(b+), S+
  - E+, K-, Jk(b+), Fy(b-), S+
  - E+, K-, Jk(b+), Fy(b+), S-

- Anti-K, -Jk^b, -Fy^b and -S were excluded from the patient's plasma by adsorption and elution studies

Anti-HLA screening

- Anti-HLA screening (ELISA) detected anti-HLA class I and II antibodies in the patient's serum

Updated Clinical Information

Bone marrow transplantation

- The patient underwent allogenic unrelated BMT
- Prior to BMT, she received myeloablative conditioning therapy according to Flu/Bu4/ATG protocol
- Flu/Bu4/ATG protocol consisted of 5 days of fludarabine (total dosage of 250 mg iv.), 4 days of busulphane (total dosage of 792 mg iv.) and 2 days of antithymocyte globulin (total dosage of 300 mg iv.)
- Before BMT, one plasmapheresis was done due to major ABO incompatibility (titer anti-A IgM 32, IgG 64)

Updated Clinical Information

Transfusion support

• Prior to BMT, she received two more O D positive E-, C^w-, K- and Fy(b-), Jk(b+), S+ red cell units that were compatible with the tube test, but positive in CAT (XM in IAT)

- Pre-transfusion Hgb=66 g/L, DAT positive
- Post-transfusion Hgb=67 g/L, DAT positive

DAT (CAT): anti-IgG (2+), -C3d (2+)

Eluate: non-specific antibody

Further Work

Donor

ABO/D: A D positive

IAT: negative

RBC	ABO	Rh	Kell	Kidd	Lewis	MNS	Duffy	P_1
Donor	A	ccD.ee, C^w neg	K-k+	Jk(a+b+)	Le(a-b+)	M+N-S+s+	Fy(a-b+)	P1+
Patient	O	CcD.ee, C^w neg	K-k+	Jk(a+b-)	Le(a-b+)	M+N+S-s+	Fy(a+b-)	P1+

Further Work

- A blood sample (pre-transfusion and post-transfusion sample) was urgently sent to the International Blood Group Reference Laboratory (IBGRL), Bristol
- After transfusion, additional testing was done in our laboratory (please see tables on next two slides)

Antibody identification panel CAT

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

AC (autocontrol): positive (mixed field appearance was not noted)
DAT (CAT): anti-IgG (2+), -C3d (2+), -IgA, -IgM, -C3c negative
EI (eluate): unidentified antibody

Antibody identification panel
Tube technology

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	RT	37C	IgG
1	+	0	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	2+	3+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	0	0	3+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	0	2+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	3+	4+	3+
5	0	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	3+	4+	3+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	0	0	2+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	0	0	1+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0	2+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	0	w	2+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0	0	1+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	w	2+
AC																				0	0	0

AC (autocontrol) negative
RT = room temperature

Further Testing Results and Interpretations

From our repeated testing

- Reactions were stronger after transfusion
- DAT became positive and a non-specific antibody was detected in the eluate
- An anti-E and anti-C^w antibody, as well as an antibody to a high-prevalence antigen were present in the patient's plasma
- Investigation stopped there as we sent a sample for an urgent investigation to IBGRL, Bristol, and we did not repeat adsorption and elution studies

Further Testing Results and Interpretations

From testing at IBGRL

- In the Preliminary Report, the presence of anti-E and anti-C^w was confirmed in the patient's plasma, an antibody to a high-prevalence antigen of anti-Yt^a specificity was found and an anti-Jk^b antibody was suspected, but further testing was needed.
- The patient's cells (from the pre-transfusion sample) were Yt(a-b+)
- An anti-Jk^b antibody was afterwards confirmed in the patient's plasma
- Four examples of Yt(a-), E-, C^w-, Jk(b-) cells were compatible with the patient's plasma and no additional antibodies were detected

Updated Clinical Information

- The patient received eight more red cell units (E-, C^w-, Jk(b-) before she was discharged from the hospital
- She was serologically monitored during transfusion support (please see the table on the next slide)
- By means of PCR-STR monitoring for chimerism, she was determined to be a 100% donor
- 3 months after BMT, only anti-C^w and -E specificity (only with the sensitive enzyme technique) were identified in the patient's plasma, and anti-Jk^b and –Yt^a were below the sensitivity of the tests

Updated Clinical Information

- Red cell units transfused and laboratory findings prior and after all red cell transfused (table).

RBC unit transfused /Transfusion event	1.	2.	3.	4.	5.	6.	7.	8.
Number of RBC units transfused	1	2	1	2	2	1	1	1
XM (IAT in CAT)	pos	pos	pos	pos	pos	pos	neg	neg
Hgb prior transfusion (g/L)	60	66	45	49	63	65	69	79
DAT prior transfusion	**neg**	pos	pos	pos	pos	pos	NT	NT
Hgb after transfusion (g/L)	71	67	56	76	74	78	86	86
DAT after transfusion	pos	pos	pos	pos	pos	pos	NT	NT
Total Bilirubin (µmol/L)	46	39	23	NT	10	12	20	27
LDH (U/L)	290	229	364	NT	189	188	268	437

NT = not tested

Conclusions

- The patient received allogenic unrelated BMT for the MDS and she immediately needed transfusion support
- Anti-C^w and anti-E alloantibodies were identified in the patient's plasma
- A suspected antibody of the anti-Yt^a specificity to a high-prevalence antigen was confirmed at IBGRL, Bristol
- Another antibody, of anti-Jk^b specificity, was suspected and confirmed to be present in the patient's plasma at IBGRL, Bristol
- She was transfused with incompatible red cell units (Yt(a+) in emergency, with no ill effects

Summary of Case Challenges

- A patient with MDS was admitted to the hospital for an allogeneic unrelated BMT and red cell units were immediately ordered
- Before transfusion, anti-E, anti-C^w and an antibody to a high-prevalence antigen (later found to be anti-Yt^a) were identified in the patient's plasma
- During the immunosuppressive myeloablative conditioning therapy prior to BMT, the patient developed an anti-Jk^b as a subsequent immune response to transfused red cell units
- She received incompatible red cell units and had a good hematological response
- After BMT, the patient was determined to be a 100% donor
- She received the last red cell unit on day +29 after BMT and was soon dismissed from hospital
- Three months after BMT, anti-Jk^b and -Yt^a were not detectable

Lessons Learned by the Case

- A patient with MDS developed multiple common antibodies (anti-E, anti-C^w) and an antibody to a high-prevalence antigen (anti-Yt^a) after 16 units of platelets were transfused a month ago, when the IAT was negative. During immunosuppressive myeloablative conditioning therapy for allogenic unrelated BMT, she developed another antibody of anti-Jk^b specificity after a transfusion of two Jk(b+) red cell units. All antibodies were developed despite the patient's diagnosis and immunosuppressive therapy
- In a patient with an antibody to a high-prevalence antigen, an underlying anti-Jk^b was very difficult to detect. As samples were urgently sent to IBGRL in Bristol, adsorption and elution studies to detect newly developed antibodies after transfusion were not done. An anti-Jk^b was detected afterwards in Bristol. From our panel results, it can be seen that reactions with two Jk(b-) RBCs were negative in the enzyme which could lead us to suspect an anti-Jk^b

Lessons Learned by the Case

- Compatible red cell units were not available, so incompatible blood was transfused (Yt(a+), E-, C^w- Jk(b-)) with no evidence of decreased red cell survival. Clinical significance of anti-Yt^a is variable, and some have been implicated in an immediate HTR. Therefore, after this case, we set up a Monocyte Monolayer Assay (MMA) to be able to predict clinical significance of unknown antibodies in the future
- In this case, the transplant donor was Jk(a-b+) and one must be aware that the patient's anti-Jk^b and anti-Yt^a could also cause acute or delayed hemolysis of the donor's Jk(b+) RBCs from the BMT and contribute to morbidity and mortality, but this was not the case, as the patient normally recovered hematopoiesis

ISBT Terminology of the YT Blood Group System

- The anti-Yt^{a} to a high frequency antigen was first found in 1956
- The antithetical antibody, anti-Yt^{b}, detects an antigen on red cells of about 8% of white people and was found eight years later
- YT, the Cartwright system, now includes 5 antigens; an inherited Yt(a-b-) phenotype has not been found.
- Cartwright is the 11th human blood group system recognized by ISBT (ISBT 011)
- The Cartwright blood group antigens are encoded by the *ACHE* gene on chromosome 7q22, which produces acetylcholinesterase (AchE)

ISBT Terminology of the Yt Blood Group System

- Two single nucleotide changes in *ACHE* are associated with Yt^a/Yt^b polymorphism: 1057C>A in exon 2 encodes His353Asn and 1432C>T, a silent mutation in exon 3 in the codon for Pro477
- Yt^a is not affected by trypsin, but is destroyed by α-chymotrypsin treatment of red cells. Papain and ficin may also destroy the antigen, but this appears to depend on the anti-Yt^a used. Yt^a and Yt^b are sensitive to disulphide bond reducing agents
- YT antigens are present on red cells from cord blood samples, but the strength of Yt^a on cord cells is weaker than that on the red cells of adults

Brief Review of the Blood Group Antibody

- Anti-Yt^a and -Yt^b are stimulated by pregnancy or transfusion, neither is ‘naturally occuring’
- YT antibodies are mostly IgG and require an antiglobulin test to agglutinate red cells
- Some anti-Yt^a bind the complement, others do not
- YT antibodies do not cause HDFN
- Anti-Yt^a has been implicated in an immediate HTR
- Many patients with anti-Yt^a have received multiple transfusion of Yt(a+) red cells with no ill effects
- For transfusion purposes, each sample of anti-Yt^a must be accessed independently. For the strong examples, Yt(a-) rare units is recommended. An MMA may also be proposed.

References

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2. Reid M, Lomas-Francis C, Olsson M. The Blood Group Antigen FactsBook, 3rd ed, Academic Press, Inc. San Diego, California, USA 2012

2. Franchini M, Gandini G, Aprili G. Non-ABO red blood cell alloantibodies following allogenic hematopoietic stem cell transplantation. Bone Marrow Transplantation 2004;33:1169-1172