CASE OF THE MONTH: MAY, 2019 (Case study by Jim Perkins)

History: A type-and-screen was received on 7/15/20-- from a 46 y.o. man with an aggressive squamous cell carcinoma (SCCA) of the genitalia who was scheduled for a radical resection of metastases to his left pubic bone and lymph nodes and soft tissue within his left pelvis. Two (2) months earlier (5/17/20--) he had received 4 units of RBCs in the course of an inguinal lymphadenectomy and chemotherapeutic limb perfusion for painful skin and soft tissue metastases to the left thigh. His antibody screen was negative at the time of that transfusion, his first ever.

On 7/15/20-- his test results were as follows:

	Anti-A	Anti-B	A1 cells	B cells	6% alb	Anti-D	<D/AHG	Interp
IS	0	3+	4+	0		4+		

Antibody Screen	
	Gel
SCI	0
SCII	vw+

Direct Antiglobulin Test (gel)			
	Polyspec.	Anti-IgG	Anti-C3
AHG	0		

Antibody Screen Cell phenotype

VS647		Rh system					Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran				
	Rh	D	C	E	c	e	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	P1	Lu^a	Lu^b		Cell	Gel
SC I	R1R1	+	+	0	0	+	0	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+		SC I	0
SC II	R2R2	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	0	+	0	0	+	+	+	+	0	+		SC II	vw+

Plasma Panel

VRA183		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		
Cell	Rh	D	C	E	c	e	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	P1	Lu^a	Lu^b		Cell	Gel
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	0	+	HLA+	1	0
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+		2	0
3	R2R2	+	0	+	+	0	0	0	+	0	+	/	+	+	+	0	+	0	0	+	+	+	+	0	0	0	+		3	vw+
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	0	+	+	0	+		4	0
5	r'r	0	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	+	0	0	+	+	+	+	0	+	HLA+	5	0
6	r"r	0	0	+	+	+	0	0	+	0	+	/	+	0	+	+	+	+	0	+	+	+	+	+	+s	0	+		6	vw+
7	rr	0	0	0	+	+	0	+	+	0	+	/	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	HLA+	7	0
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+s	0	+	HLA+	8	0
9	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	+	0	0	+		9	0
10	rr	0	0	0	+	+	0	0	+	0	+	/	+	0	0	+	0	0	+	0	+	+	+	+	+	0	+	HLA+	10	0
11	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	+	0	+		11	0
Patient																													AC	0

Se;ected Cell Panel

VRB181		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		
Cell	Rh	D	C	E	c	e	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	P1	Lu^a	Lu^b		Cell	Gel, 30'
15	R2R2	+	0	+	+	0	0	+	+	0	+	/	+	+	+	0	+	+	+	0	0	+	+	+	+	+	+		15	1+
16	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+		16	1+

Extended Phenotype

	Rh system				Kell				Kidd		Duffy		Lewis		MNSs													
	C	E	c	e	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	S	s	M	N	P1	I	H	A_1						
Patient	+	0	+																									

Questions/discussion:

What is the specificity of the patient's antibody? Is it proven? Would this problem meet the CDC/AABB criteria for a delayed hemolytic transfusion reaction (DHTR)?

This patient has an alloanti-E that meets all the criteria for proof (3 E-pos cells reactive, 3 E-neg cells nonreactive, "everything else" ruled out, patient is E neg). This case does not meet the criteria for a DHTR as the new antibody was detected more than 28 days after transfusion.

The patient received 6 units of RBCs over 3 days, 7/15, 7/16, and 7/17/20--. Another specimen received on 8/2/20-- yielded the following test results.

	Anti-A	Anti-B	A1 cells	B cells	6% alb	Anti-D	<D/AHG	Interp
IS	0	4+	4+	0		4+		

Antibody Screen	
	Gel
SCI	2+
SCII	0

Direct Antiglobulin Test (gel)			
	Polyspec.	Anti-IgG	Anti-C3
AHG	0		

Antibody Screen Cell phenotype

VS654		Rh system					Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran				
	Rh	D	C	E	c	e	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	P1	Lu^a	Lu^b		Cell	Gel
SC I	R1R1	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	0	0	+		SC I	2+
SC II	R2R2	+	0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	0	+	0	+		SC II	0

Plasma Panel

VRA184		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		
Cell	Rh	D	C	E	c	e	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	P1	Lu^a	Lu^b		Cell	Gel
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+		1	0
2	R1R1	+	+	0	0	+	0	0	+	0	+	/	+	+	+	0	+	0	0	+	+	0	0	+	+	0	+		2	0
3	R2R2	+	0	+	+	0	0	0	+	0	+	/	+	0	+	+	0	+	0	0	0	+	+	+	0	0	+	HLA+	3	0
4	Ror	+	0	0	+	+	+	0	+	0	+	/	+	0	0	+	0	0	0	0	+	+	0	+	+	0	+	HLA+	4	1+
5	r'r	0	+	0	+	+	0	0	+	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+s	0	+		5	0
6	r"r	0	0	+	+	+	0	0	+	0	+	/	+	+	0	+	+	+	0	+	+	+	+	0	+	0	+		6	0
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+s	0	+	Co^b+	7	2+
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+		8	0
9	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+		9	0
10	rr	0	0	0	+	+	0	0	+	0	+	/	+	+	0	+	+	0	0	+	+	+	+	0	+	0	+		10	w+
11	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+s	+	+		11	2+
Patient																													AC	0

Extended Phenotype

	Rh system				Kell				Kidd		Duffy		Lewis		MNSs							
	C	E	c	e	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	S	s	M	N	P1	I	H	A_1
Patient	(+)	(0)	(+)		0																	

Questions/discussion:

What is the specificity of the patient's antibody(ies) now? What antibody(ies) is proven? What other tests would you like to do?

This patient now has an alloanti-K that again meets all the criteria for proof (3 K-pos cells reactive, 3K-neg cells nonreactive, "everything else" ruled out, patient is K negative). Surprisingly, the anti-E demonstrated just 18 days earlier appears to be completely gone as shown by 2 non-reactive R2R2 cells! In addition to the anti-K there are 2 K-, E- cells (#'s 4 and 10) reacting weakly. Looking at the phenotypes of these 2 cells note that both are Jk(a+), although one is Jk(a+b-) and the other is Jk(a+b+). However, 3 other Jk(a+b-) cells are NOT reacting, so anti-Jk^a was "ruled out". The appearance of a new antibody 17 days after transfusion suggests a DHTR, and one would like to determine whether any of the recently-transfused units were K positive.

Retained segments were retrieved from the 6 units of RBCs transfused between 7/15 and 7/17/20--. Two (2) were K-positive, both of which were given on 7/16.

Questions/discussion:

Does this event meet the criteria for a DHTR?

This event appears to represent a DHTR as the new antibody was detected within 28 days after the last transfusion, although again, the CDC/AABB criteria for a "definitive" DHTR cannot be met (as discussed in the April case) because the DAT is negative. We would also like to see evidence of hemolysis. Although the patient's hemoglobin fell after transfusion he had complications with his large wound so we cannot say he was hemolyzing by that criterion. Also we would not be surprised to see a small bump in his bilirubin or LDH levels due to lysis of tissue hematomas. The facts that no K-positive donor cells are detected in the K phenotyping and that the DAT is negative suggests that the K-positive donor cells have been destroyed, but these results are not part of the CDC/AABB criteria.

The patient continued to have severe pain from the metastases in his left leg, and necrotic metastases to his skin were open and infected. Therefore, it was decided to attempt a "hindquarter amputation" (removal of the entire leg and much of the left half of the pelvis) for palliation. In preparation for the surgery 2 units of RBCs were requested and a specimen was submitted on 8/19/20--, 17 days after the last one. He had not had transfusion in the interim. The following results were obtained:

Direct Antiglobulin Test (gel)			
	Polyspec.	Anti-IgG	Anti-C3
AHG	0		

Selected cell panel

VRA185		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		
Cell	Rh	D	C	E	c	e	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	P1	Lu^a	Lu^b		Cell	Gel
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	0	0	+	0	+	+	+	+		1	0
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	+	0	+s	0	+	HLA+	2	w+
3	R2R2	+	0	+	+	0	0	0	+	0	+	/	+	0	+	0	+	+	0	+	0	+	+	0	0	0	+		3	0
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	0	0	0	+	+	+	+s	0	+		4	1+
5	r'r	0	+	0	+	+	0	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+		5	1+
6	rr	0	0	0	+	+	0	0	+	0	+	/	+	+	0	+	0	+	0	0	+	+	0	+	+	0	+		6	w+
7	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	0	0	0	+	+	0	+	0	+		7	w+
8	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+s	0	+		8	0
9	r"r	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+		9	0
Patient																													AC	0

Extended Phenotype; Jk testing was performed on the current (8/19) specimen

	Rh system				Kell				Kidd		Duffy		Lewis		MNSs							
	C	E	c	e	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	S	s	M	N	P1	I	H	A_1
Patient	(+)	(0)	(+)		(0)						0	+										

Questions/discussion:

What antibodies does the patient have now? Are any new antibody specificities proven? What other tests would you like to do? Is this another DHTR?

The patient still has detectable anti-K, and now the previously suspected anti-Jk^a is proven. The mystery of the disappearing anti-E continues! This workup really just provides more information about the DHTR detected on 7/15/20--, namely that there were 2 new antibodies, albeit anti-Jk^a could not be proven on the 8/2 specimen. We would like to know the Jk^a phenotype of the 6 units transfused in July, but the segments from those units were discarded after typing the units for K.

What phenotype of RBCs would you reserve for the patient? What percentage of Caucasian donor would be expected to have that phenotype? Is there anything else you would like to do?

The patient should ideally receive RBCs lacking K, Jk^a, and E antigens. Fifteen percent (15%) of Caucasians are expected to have this phenotype. The 2 units of RBCs requested are unlikely to be enough for this huge surgery involving multiple large blood vessels and an operative field that had been operated before as well as irradiated. In consultation with the anesthesiologist the blood bank decided to increase the order to 10 units.

The patient underwent hindquarter amputation on 8/21/20--. After a couple of hours it became obvious that the 10 units of compatible RBCs would not be sufficient. The blood center was contacted for additional units, but since their stock of E-, K-, and Jk^a-negative units had already been depleted the blood bank was informed that the order could not be filled for several hours. Luckily the hospital is part of a 4-hospital system, all within 30 minutes travel time. Typing of the hospital's remaining inventory of group B and O RBCs for this phenotype was instituted, and the other 3 hospitals were enlisted in the same search. Additional units were identified within the system and delivered emergently. The patient ended up using 18 units of RBCs, 1 unit of Platelets, Apheresis, 8 units of plasma, and 3 pools (15 units total) of cryoprecipitate. Two additional units of RBCs were transfused the following day (post-operative day 1).

On post-operative day 2 additional platelets were requested and a new patient specimen was submitted. The antibody screening test on this specimen was negative.

Questions/discussion:

Why do you think the antibody screen is negative? Could he have been transfused differently?

The patient had received a massive transfusion of almost 2 blood volumes. This is sufficient to have "washed out" his antibodies, so it was probably unnecessary to have given the last 8 RBCs as "antigen negative". However if incompatible units had been used there may have been delayed hemolysis after the operation.

On 9/15/20-- (23 days later) an additional unit of RBCs was requested, and a new patient specimen was received. The antibody screening test was still negative. The patient died of respiratory failure due to pulmonary metastases 2 weeks later, about 14 months from diagnosis.

Why do you think the patients antibody screen is still negative?

We can only guess that his overall condition, including chemotherapy, radiation, and perhaps massive transfusion (by transfusion-related immunomodulation or TRIM), had suppressed his ability to rapidly synthesize a detectable level of his antibodies.

Take home points:

In a situation of ongoing transfusion of an immunocompetent patient the antibody situation can change rapidly, justifying the requirement for a new patient blood specimen every 3 days.

In very massive transfusion of patients with antibodies it may not be necessary to provide all RBCs as lacking the corresponding antigens.