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

A 74 year old woman fell while playing tennis in Italy sustaining a femoral neck fracture. Three days later, after returning to the U.S., she was admitted to the hospital where she underwent a total hip arthroplasty on the third hospital day. The patient had delivered 3 children. She had pernicious anemia for which she received monthly vitamin B12 injections, and her admission hemoglobin (hgb) was 12 G/dL. On post-operative day 3 her hgb was 9.0, and because of concern that her anemia might compromise her rehabilitation she was transfused 2 units of RBCs, finishing early on the 4th post-op. day, and was discharged. Pretransfusion testing performed on admission and on post-op. day 3 demonstrated that she was group A, Rh positive and her blood group antibody screen by the "gel" method was read as negative on both occasions. Of note, the patient was 5'2" tall and weighed 48.5 KG. Her pulse rate hours before and after transfusion was 80/min. the pulse did increase to 102 at the end of the first unit, but there was no fever. The following day she was discharged to a rehabilitation facility with a hgb of 11.8.

Thirteen days after the transfusion the patient reported fatigue, loss of appetite, and brown urine at the rehabilitation facility, and she was re-admitted to the hospital. Her hgb was 9.8. The urine appeared clear and straw colored; there was "3+ blood" but only 5-10 RBCs/hpf. The total bilirubin was 1.8 mg/dL and dropped to 0.8 the following day (it was 1.4 on admission for fracture). The following immunohematologic test results were obtained:

ABO and Rh Typing

<A	<B	A1 cells	B cells	6% alb	<D	<D/AHG	CCC	Interp
4+	0	0	4+		4+			

Antibody Screen

	GEL
OI	0
OII	4+

Direct Antiglobulin Test (tube)

	Poly	IgG	<C3
AHG	0		
CCC	2+		

Initial panel

Lot# 8RA186		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	+	+	0	+	C^w	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	+		3	4+
4	Ror	+	0	0	+	+	0	0	+	0	+	+	+	0	0	+	+	+	0	0	0	+	+	0	+	0	+		4	3+
5	r'r	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	+	+	0	+	+s	0	+		5	2+
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+		6	$3+^s$
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	0	+	+	0	+	0	+		7	3+
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0	+	0	+		8	3+
9	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+		9	3+
10	rr	0	0	0	+	+	0	0	+	0	+	+	+	0	0	+	0	+	+	0	0	+	+	+	+s	0	+		10	3+
11	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	+	0	0	+		11	0

Questions/discussion:

What antibody(ies) do you think is present? Is more work needed to prove this hypothesis? What do you think is going on here clinically? What testing would you like to do to investigate this possibility?

The patient appears to have anti-c (all c-positive cells reacting, 4 c- negative cells not reacting) and possibly anti-E (note stronger reactions with R2R2 and r'' cells) which is new since the previous transfusion. The possibility of anti-E can be proven by testing E-pos, c-neg cells, the most available of which are RzR1 cells. We also need to do an extended Rh phenotype to demonstrate that the patient lacks the c and E antigens.

The fact that the patient has new antibodies after transfusion as well as evidence of hemolysis in the form of a fall in the hgb level and signs of hemolysis (brown urine, hemoglobinuria on UA, hyperbilirubinemia) suggests a delayed hemolytic transfusion reaction DHTR. Typical workup would include typing of retained segments of the transfused units for c and E antigens, and a repeat antibody screen on the pre-transfusion specimen.

The following additional workup was performed:

Selected cells

		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	RzR1	+	+	+	0	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	+	0	+		1	4+
2	RzR1	+	+	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+		2	4+
3	RzR1	+	+	+	0	+	0	0	+	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+s	0	+		3	4+
4	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+		4	0

Extended Phenotype (controls not shown)

	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	+																							
Unit #1		+	+																								
Unit #2		+	0																								

Transfusion reaction workup

	ABO and Rh Typing						DAT (tube)	Antibody screen, gel		AHG crossmatch (saline/tube IAT w 4 drops plasma)					
	<A	<B	A1 cells	B cells	<D	Interp	Poly	SCI	SCII	Donor 1			Door 2		
										IS	37°, 30'	AHG	IS	37°, 30'	AHG
Pre-transfusion specimen	4+	0	0	4+	4+		0	0	w+						
Post-transfusion specimen	4+	0	0	4+	4+		0	0	4+	0	2+	4+	0	2+	4+

Selected cells, pretransfusion specimen

		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		PEG
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	AHG
1	RzRz	+	+	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	+	0	+	+		1	0
2	rr	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0	0	+		2	2+

Questions/discussion:

Is the hypothesis regarding the patient's post-transfusion antibody specificity proved? What about the hypothesis regarding the DHTR? Are there any surprises in this workup? Do you think that this transfusion was necessary?

The additional workup confirms the fact that after transfusion the patient had allo-anti-c and anti-E (I.E. 3 c-pos/E-neg cells reactive, 3 E-pos/c-neg cells reactive, 3 c-neg/E-neg cells non-reactive, other antibodies ruled out, patient lacks c and E antigens).

Transfusion reaction workup also demonstrates that both of the previously transfused RBCs were E-positive and one was c-positive and therefore were capable of stimulating the corresponding antibodies. As we would expect, both units are incompatible with the post-transfusion specimen, but unfortunately they were not crossmatched with the pre-transfusion specimen. Of note however, the repeat antibody screen on the pretransfusion specimen was weakly positive; prior to transfusion it had been resulted as negative, and the units had been crossmatched by an "immediate spin" test only. Note that the rr cell reacts but the RzRz cell does not, so presumably the patient already had a weak anti-c, but the anti-E was not detectable. So this DHTR was probably due to an error in reading the manual gel antibody screening test, and was therefore avoidable. Nonetheless, the anti-c was too weak initially to immediately cause overt hemolysis or even a fever. The increase in her pulse rate could have been due to the incompatibility but could equally be due to a degree of volume overload in this small patient. Also note that the anti-c in the pre-transfusion specimen reacted more strongly with PEG enhancement than in the gel test used for routine screening. Finally, DHTRs do not usually cause symptoms or signs other than a fall in hemoglobin in the presence of a new antibody, but this patient had hemoglobinuria.

To summarize, this patient didn't show evidence of a hemolytic reaction at the time of the transfusion in spite of the fact that the first unit was c-positive, but over the next 13 days she clearly had hemolysis in association with an increase in the strength of the anti-c and a new anti-E. It's interesting to look at the CDC/AABB hemovigilance definition of a DHTR in relation to this case. Note that when the positive antibody screen was discovered the DAT was negative, probably because there were no donor cells left for the stronger anti-c and new anti–E to bind to. However according to the CDC/AABB definition a "Definitive" DHTR requires a positive DAT; this case would only fit the criteria for a "Probable" DHTR. Moreover, whether definitive or probable the criteria would only apply to the anti-E. If the anti-c had been the only antibody present and she had only hemolized after it had become stronger, because it was present at the time of transfusion the criteria for diagnosis of a DHTR would not have been met at all. But could we call this an immediate hemolytic transfusion reaction? Clearly the hemolysis did not occur immediately but was present after some number of days had passed.

In the author's experience many obvious DHTRs do not fit the CDC/AABB criteria for a "Definitive" DHTR because of the requirement for a positive DAT, and the criteria for IHTRs suffer from a similar problem. Once the donor cells are gone, the DAT cannot be positive!

This reaction was avoidable in an additional sense that the transfusion was probably unnecessary! The pre-transfusion hgb level was 9.0 G/dL and the post-transfusion hgb was near the lower limit of normal at 11.8. At the time of transfusion the physician's notes explicitly identify the patient's ability to fully participate in physical rehabilitation as an indication for the transfusion. This case is over 10 years old at the time of this writing, and the transfusion practices illustrated are out of date. A randomized trial (Carson, FOCUS trial, NEJM 2011) of restrictive (hgb < 8 G/dL) versus liberal transfusion in patients identical to this one showed no difference in functional outcome and, by inference, ability to undergo rehabilitation.

Take home points

When a DHTR is suspected, repeat testing of a pre-transfusion specimen is an important quality control measure to detect whether the reaction was avoidable.

Weak antibodies present at the time of transfusion may not cause immediate hemolysis, but may only cause hemolysis in a delayed fashion. (This conclusion assumes that hemolysis would still have occurred due to the anti-c without the appearance of anti-E.)

The DAT is often negative at the time of detection of a DHTR, and this fact is not recognized by the CDC/AABB criteria for a DHTR.

One way to avoid negative outcomes to transfusions is to follow restrictive transfusion practices.

The patient was not transfused after being readmitted. The following figure shows the time course of the patient's hemolytic reaction.