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CASE SET #17

An Immunohematology Case:

Alloimmunization to a Complex Rh Antigen, f

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Clinical Laboratory Investigations

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LEARNING OBJECTIVES

Upon completion of reading the case, the learner will be able to:

1. State the characteristics and frequency of the f antigen and characteristics of the anti-f antibody.

2. Identify an anti-f alloantibody when given panel reaction results.

3. Differentiate anti-f from anti-e and anti-c and explain how alloimmunization to f can occur.

4. Describe the best method to use when crossmatching a patient with an anti-f alloantibody.

Alloimmunization to a Complex Rh Antigen, f

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CASE PRESENTATION

A 70-year old male arrived at the emergency department (ED) on March 11, 2013. He presented with sharp pains in his right abdominal quadrant and nausea. He had a history of hypertension, hyperlipidemia and renal disease. Past surgeries included a coronary artery bypass in 2012 and multiple back surgeries. He denied any alcohol, tobacco or illicit drug use. Of note was his most recent hospitalization on February 20, 2013 during which he was treated for a subdermal hematoma and also received four units of packed red blood cells (RBC) and one unit of platelets. This included two A Rh positive RBC units, two A Rh negative RBC units and one O Rh positive platelet unit.

The patient's vitals upon admission were unremarkable, and he rated his pain at 8 on a scale of 1-10. The admitting physician ordered a complete metabolic panel (CMP), complete blood count (CBC) and a computed tomography (CT) scan of his abdomen. The chemistry and hematology results are shown in Tables I and II, respectively.

Table I. Patient's Chemistry Results				
	3/11/2013 Admission	3/13/2013 Post-surgery	3/15/2013 Discharge	Reference Range
Calcium	9.7	8.5	8.8	8.5-10.5 mg/dL
Glucose	108	120	97	60-110 mg/dL
BUN	38	35	22	7-18 mg/dL
Creatinine	1.54	2.03	1.48	0.4-1.2 mg/dL
Total Protein	7.7			6.4-8.3 g/dL
Albumin	3.6			3.4-5.0 g/dL
T. Bilirubin	0.8			0.1-1.0 mg/dL
ALK PHOS	92			38-120 U/L
AST	20			10-42 U/L
ALT	20			5-50 U/L
Sodium	136	141	140	135-145 mmol/L
Potassium	4	3.7	3.7	3.5-5.2 mmol/L
Chloride	99	104	103	95-108 mmol/L
CO_2	26	27	28	21-32 mmol/L
GFR	45	33	47	>60 mL/min/1.73m^2

BUN- Blood urea nitrogen, T. Bilirubin- Total Bilirubin, ALK PHOS- Alkaline phosphatase AST- Aspartate transferase, ALT- Alanine transferase, CO_2- Carbon dioxide, GFR- Glomerular filtration rate

Table II. Patient's Hematology Results					
	3/11/2013 Admission	3/13/2013 Post-surgery	3/13/2013 Post-transfusion	3/15/2013 Discharge	Reference Range
White Blood Cell Count(WBC)	9.10	4.6	5.2	4.9	3.8-11.0 K/uL
Red Blood Cell Count(RBC)	3.44	2.3	2.9	2.91	4.2-5.7 M/uL
Hemoglobin (Hgb)	10.3	6.9	8.4	8.7	13.7-16.7 g/dL
Hematocrit (Hct)	31.5	20.7	26.0	26.4	40.0-50.0 %
Mean Cell Volume (MCV)	91.5	92.1	90.2	90.7	80.0-100.0 fL
Mean Cell Hemoglobin (MCH)	30.0	30.6	29.1	29.8	27.0-34.0 pg
Mean Cell Hemoglobin Concentration (MCHC)	32.8	33.2	32.3	32.9	32-36 g/dL
Red Cell Distribution Width (RDW)	16.8	18.0	16.7	16.3	11.0-15.5 %
Platelet	351	204	217	224	150-400 k/uL
Mean Platelet Volume (MPV)	6.70	6.7	6.1	6.9	6.0-11.0 fL
Neutrophils	82.3	74.3	74.8	65.0	38.0-70.0 %
Lymphocytes	7.9	11.6	9.2	15.5	21.0-49.0 %
Monocytes	5.3	8.9	9.3	10.8	3.0-11.0 %
Eosinophils	4.1	4.9	6.6	8.3	0.0-7.0%
Basophils	0.4	0.3	0.1	0.4	0.0-2.0 %

The patient's CMP revealed an increased blood urea nitrogen (BUN) and creatinine with a low glomerular filtration rate (GFR). These findings could be attributed to his history of renal failure. His CBC revealed a low hemoglobin (10.3g/dL) and hematocrit (31.5%), however these were not critical at the time. His white blood cell count (WBC) was toward the high end of the reference range, and neutrophilia was apparent in the differential. Although the laboratory results were overall unremarkable, the CT scan of his abdomen revealed inflammation in his lower right abdomen. He was diagnosed with appendicitis and underwent an immediate laparoscopic appendectomy.

There are no clinical laboratory tests specific for the diagnosis of appendicitis. However, results in relation to the appendicitis can include leukocytosis with neutrophilia, pyuria, hematuria and an increase in C-reactive protein (CRP). [1] Predictive signs and symptoms associated with appendicitis include right-lower-quadrant pain, abdominal rigidity, and migration of pain from the periumbilical region to the right lower quadrant. It can also be associated with nausea, vomiting and anorexia. The diagnosis for appendicitis is often confirmed in adults using a CT scan. Indications of appendicitis include findings of a distended appendix, a thickened appendiceal wall, and periappendiceal inflammation.[1]

Two days following the appendectomy the patient reported feeling fatigued; laboratory results post-surgery are shown in Tables I and II. The results revealed that he had lost a significant amount of blood, which was indicated by a hemoglobin of 6.9 g/dL and a hematocrit of 20.7%. There were no identified indications of bleeding and the patient's vitals were normal, however given his history of coronary artery disease, the physician ordered a type and screen and two units of RBCs. The patient typed A Rh positive, with a negative antibody screen using the Micro Typing Systems (MTS) gel technology. Two A Rh positive RBC units were transfused on March 13, 2013. Two days post-transfusion, March 15, 2013, the patient's hemoglobin and hematocrit had increased to 8.4 g/dL and 26% respectively (shown in Tables I and II), and he was discharged.

The patient returned to the ED on April 23, 2013. This time he was diagnosed with right carotid stenosis and was scheduled to undergo a carotid endarterectomy the next day. The physician ordered a type and screen. The results are shown in Table III.

Table III. Patient's Type and Screen 4/23/13	
ABO	A
Rh	Positive
Screen Cell I	0
Screen Cell II	0
Screen Cell III	3+
AC	Negative
DAT	Negative

AC- Auto control, DAT- Direct anti-globulin test

The patient again typed A Rh positive, however his antibody screen was now positive. Both the auto control (AC) and the direct antiglobulin test (DAT) were negative, indicating his cells were not coated with antibody and no autoantibody was present. This suggested the presence of an alloantibody. An antibody identification panel (AB ID) was performed using the patient’s serum, and the results are displayed in Table IV. This panel showed antibody specificity to f antigen.

Table IV. Patients antibody ID panel results

			Rh								Kell						Duffy		Kidd			Lewis		MNS				P	Lutheran		
cell #	Rh-hr	Donor Number	D	C	E	c	e	f*	Cw	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	P_1	Lu^a	Lu^b	MTS
1	R1wR1	300514	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	+	0	0	0	+	+	0	+s	0	+	0
2	R1R1	305843	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	0	0	+	+	0	+	0
3	R2R2	305876	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	+	0
4	Ror	311531	+	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	3+
5	r' r	308880	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	+	+	0	0	+	2+
6	r" r	71221	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	0	+	0	+	+	0	0	+	3+
7	r r	311820	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	3+
8	r r	113366	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+s	0	0	+	3+
9	r r	311797	0	0	0	+	+	+	0	0	0	+	0	+	/	+	0	+	+	+	+	0	+	+	0	+	0	0	0	0	+	3+
10	r r	304346	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	0	+	3+
11	R1R1	309091	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	0	0	0	+	0

In order to confirm the specificity of the alloantibody to f, the patient was antigen typed for the Rh antigens C, E, c, and e (Table V).

Table V. Patient's Antigen Typing	
Antisera	Results
C	3+
E	0
c	0
e	3+
Phenotype: D, C, e	

The patient's phenotype was D,C,e. The most likely genotype, given his phenotype, is R1R1 (DCe/DCe), which is the genotype of approximately 18.5% of Caucasians and 22.3% of Rh-positive Caucasians.[2]

The f (ce) Antigen

The f(ce) antigen is a Rh antigen that is present in 65% of Caucasians, 92% of Blacks, and 12% of Asians.[3] It was previously considered a compound antigen, however it is now believed to be a single entity resulting from conformational changes in the Rhce protein.[2] The f antigen is a result of positional effects of the alleles on the chromosomes. *Cis* products of these genes are only expressed when the alleles are on the same haplotype. Therefore, the f antigen is only expressed when c and e alleles are on the same haplotype, or *cis*. For example, individuals with dce and Dce haplotypes will express the c, e and f antigens.[4] Individuals with c and e expression in the *trans* position, as seen with the genotype DcE/DCe, will express both c and e antigens, but will not express the f antigen. Interestingly, the f antigen can be expressed in some individuals who have a Dc- phenotype, however the expression of f is greatly reduced.

Like other Rh antigens, f is strongly expressed at birth and is not destroyed by enzymes or chemicals such as dithiothreitol (DTT) or acid.[3,4]

Anti-f

Anti-f is a weakly reacting antibody that reacts with the f(ce)antigen and is often found with other antibodies.[2] Characteristic reactions based on genotype are shown in Table VI.[5] It is typically an alloantibody present after exposure to antigens not present on one's own RBCs, typically found in cases of transfusion or with pregnancy, however cases of autoantibodies have been reported. As with other antibodies, it is possible to be exposed to f positive red blood cells, yet not produce an antibody. The anti-f antibody is predominately of the IgG immunoglobulin class, does not show dosage, and does not bind complement, however occasional findings of anti-f IgM have been reported.

Table VI. Anti-f Reactions with Common Genotypes

	Genotype	Reaction
R1r	DCe/dce	+
R1R1	DCe/DCe	0
R2r	DcE/dce	+
R2R2	DcE/DcE	0
R1R2	DCe/DcE	0
rr	dce/dce	+
R0R0	Dce/Dce	+

The best techniques for *in vitro* analysis include room temperature, indirect antiglobulin testing (IAT), and enzyme treatment. The optimal temperature for reactivity of anti-f is 37°C. As with other Rh antigens, antigen modification enzyme treatment will enhance the reactivity of the antigen. Anti-f is clinically significant and has been implicated in cases of mild or delayed hemolytic transfusion reactions and hemolytic disease of the newborn.[3] Since predominately of the IgG class, it results in extravascular hemolysis.[2]

Because f antiserum is not commercially available, it is impossible to type for the f antigen. If crossmatching an individual with alloanti-f, other simple methods should be applied. Blood donors who are either c-negative or e-negative are f-negative by definition. Thus, units that type negative for c or e are considered f-negative and should be selected for further

compatibility testing. Since e is present in approximately 98% of units, and c in 80% of units (Caucasian statistics), typing for and crossmatching c-negative units would be more efficient and likely to result in compatibility. Furthermore, most people who have alloanti-f will be Rh-positive. This is due to genotype frequency in that most Rh-negative individuals, around 91%, have at least one dce haplotype (gene) and by definition, the f antigen.[2] Due to the high incidence of the f antigen in Rh-negative individuals, type-specific blood should be given for transfusion when possible. Most Rh-negative units are rr (dce/dce), while approximately 48.8% of Rh-positive units have a dce haplotype.[2]

PATIENT OUTCOME

Given the patient's positive antibody screen, he was MTS crossmatched and compatible with two A Rh positive, c- units, however he did not need a transfusion during the carotid endarterectomy procedure. He was discharged on April 26, 2013 in stable condition with a 3.35 M/ul RBC count, 10 g/dL hemoglobin, and 30.3% hematocrit. The patient returned again on August 6th with a diagnosis of lumbar stenosis and was to undergo another surgery. His screen was still showing alloanti-f with the same strength of reactivity. Overall, between February 13, 2013 and March 13, 2013 he received four A Rh positive RBC units and two A Rh negative RBC units, which resulted in a positive alloantibody screen on April 23, 2013.

DISCUSSION

Given the patient's phenotype, C+, E-, c-, e+, his red cells were f negative. Most, approximately 91%, Rh-negative units are positive for the f antigen.[2] This begs the question of how many Rh-positive individuals are inadvertently exposed to f when transfused with Rh-negative blood. Approximately 48.8% of Rh-positive individuals have one dce or Dce haplotype and, therefore, will not elicit an immune response. However, the remainder of Rh-positive individuals can develop the alloantibody upon exposure to the f antigen either through

pregnancy or transfusion.[2,3] In this case, the man was Rh-positive, but was given two units of Rh-negative RBCs. This could have sensitized him to the f antigen; however, multiple units were transfused within the two-month period prior to the development of the alloantibody, so any one of the units could have stimulated an immune response. This antibody is of clinical significance, and the patient should continue to receive c-negative units if more transfusions are deemed necessary, even if the antibody titer becomes undetectable in future blood work-ups.[3] It is also important to consider at what level of hemoglobin a patient is deemed necessary for transfusion and to not over-transfuse. It may also be helpful to look at any history the patient may have of a normally low hemoglobin/hematocrit level. Transfusing only when medically necessary will limit the patient's exposure to potentially foreign antigens, and prevent sensitization.

REFERENCES

1. Paulson EK, Kalady MF, Pappas TN. Clinical practice. Suspected appendicitis. N Engl J Med. 2003 Jan 16;348(3):236-421.

2. Harmening DM. The Rh Blood Group System In: Modern Blood Banking and Transfusion Practices. Sixth ed. Philadelphia, PA: F. A. Davis Company; p. 163-4.

3. Reid ME, Lomas-Francis C. Rh blood group system In: The Blood Group Antigen: *Factsbook*. Second ed. Amsterdam: Boston: Elsevier/Academic Press; 2004; p. 139-40.

4. Jator EK, Pedde E. Anti-f in a 24-year-old male: A case study. Clin Lab Sci. 2010 Spring;23(2):68-71.

5. Chow PK, Tsoi WC. Antibody against compound antigen ce(f). JHKMTA. 1997/98;7:19-21.