Warm autoantibody or drug-dependent antibody? That is the question!

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Clinical Case Presentation

Two units of leukocyte-reduced red blood cells (LRBCs) were ordered for a 58-year-old Caucasian woman whose hemoglobin was falling. She had undergone a cholecystectomy 2 weeks earlier. Her laboratory test results are noted in Table 1.

Table 1. Laboratory test results

Result	Day 1	Day 2
Hb (g/dL)	7	5.1
Bilirubin, total (mg/dL)	1.2	4.3
LDH (IU/L)	847	2305
Reticulocyte count	6%	NA

NA = not available

Immunohematologic Evaluation and Results

Results of initial pretransfusion testing showed her RBCs to be group O, D+. An antibody detection test performed by gel technology was positive (2+) with both reagent screening RBCs. An antibody identification panel was also tested by gel and similar positive reactivity (2+) was seen with all panel RBCs. The hospital technologist performed a DAT by the gel test. This was positive (4+) with anti-IgG while the saline control was negative. The cards available at the hospital did not include polyspecific antihuman globulin (AHG) or anti-C3. At this point, the hospital technologist performing the testing was convinced that this patient had a warm autoantibody and sent a sample to the Immunohematology Reference Laboratory (IRL) for further evaluation to determine whether the patient had any underlying alloantibodies. The clinician was informed of the serologic results and that there would be a delay in obtaining blood because the patient's sample was being sent out.

Panel positive reactions with equal reactivity are most likely the result of an autoantibody or an alloantibody to a high-prevalence antigen present on all reagent RBCs tested. An autologous control will help differentiate an autoantibody from an alloantibody. If the autologous control is negative, one is most likely dealing with an antibody to a high-prevalence antigen, but if positive with equal or stronger reactivity (2+ to 4+), an autoantibody is most likely. Alternatively or in addition to the autologous control, a DAT may be performed. This may be the method chosen in laboratories routinely using gel or solid phase for antibody detection and identification. It is important to remember, however, that the autologous control and DAT are different tests. An autologous control includes patient serum whereas a DAT only tests patient RBCs. In most cases, if the DAT is positive, the autologous control will also be positive. If the patient's RBCs are sensitized with IgG or C3 in vivo, the autologous control will be positive because this occurred before incubation. However, there are situations when only the autologous control will be positive because the antibody reactivity is method-dependent. The IgG coating of patient RBCs in this case suggests a warm autoantibody.

Underlying alloantibodies are reported in 12 to 40 percent of patients with warm autoantibodies.[1] Review of the patient's clinical history showed she had received a transfusion of two units of RBCs 20 years earlier, and she delivered three children.

The results obtained in the IRL using test tube methods are in Table 2.

According to this IRL's policy, an enzyme-treated (ficin) antibody identification panel is first tested when a warm autoantibody is suspected. Patient RBCs are treated with ZZAP (ficin and DTT) before performing

Table 2. Antibody identification panel*: results of testing serum from the patient

	Rh						MNS				Lu		P	Lewis		Kell		Duffy		Kidd		Saline		
Cell	**D**	**C**	**E**	**c**	**e**	**f**	**M**	**N**	**S**	**s**	**Lu^a**	**Lu^b**	**P$_1$**	**Le^a**	**Le^b**	**K**	**k**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**IS**	**37°C**	**IAT†**
1	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	0	2+
2	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	+	0	+	+	+	0	0	0	1+
3	0	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	+	0	0	+	0	0	1+
4	+	+	0	0	+	0	+	0	+	+	0	+	0	0	0	0	+	+	+	+	+	0	0	2+
5	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	1+
6	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	2+
7	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	0	0	1+
8	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	0	1+
9	+	+	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	+	+	0	0	0	1+
10	+	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	1+
AC																						0	0	4+

AC = autologous control

* In-house prepared RBC panel

†Anti-IgG, ImmucorGamma, Inc., Norcross, GA

an autologous adsorption. Alternatively, if allogeneic adsorptions are performed, these reagent RBCs are pretreated with ficin. Initial screening of a ficin-treated panel ensures that the autoantibody is directed against an antigen present on ficin-treated RBCs, and, therefore, adsorptions should be effective. The ficin panel was 3+ with all RBCs, and the autologous control was 4+ reactive. Enhanced reactivity is consistent with a warm autoantibody.

Results of the test tube DATs performed in the IRL are in Table 3. It is important to include a control to rule out spontaneous agglutination when positive reactivity is seen with all reagents.

The strongly positive DAT with IgG and complement (C3b,C3d) is most likely caused by a warm autoantibody, especially in light of the serum results. Approximately 50 percent of positive DATs in patients with warm autoimmune hemolytic anemia (WAIHA) show both IgG and complement coating the RBCs.[1]

In an effort to ensure that blood was available as quickly as possible for this patient, the IRL technologist started autologous adsorptions because the patient had not been recently transfused. One milliliter of RBCs was saved for the eluate. While the patient's serum was incubating at 37°C with the first set of the patient's ZZAP-treated RBCs, a rapid acid eluate (ELU-KIT II, ImmucorGamma, Inc., Norcross, GA) was performed. To the technologist's surprise, the eluate was negative!

Table 3. DAT results

	IS	10 min. RT incubation
Polyspecific AHG*	4+	NT
Anti-IgG†	4+	NT
Anti-C3b,C3d‡	1+	2+
Saline control	0	0

IS = initial spin, RT = room temperature, NT = not tested

*Polyspecific AHG, Ortho Clinical-Diagnostics, Inc., Raritan, NJ

†Anti-IgG, ImmucorGamma, Inc.

‡Anti-C3b,C3d, ImmucorGamma, Inc.

The IRL technologist performing the work immediately called the referring institution to notify them of the results of the eluate. A negative eluate is highly suggestive of a drug-dependent antibody. Drug-dependent antibodies will not react, even if eluted from patient RBCs, because the putative drug must be present when testing the eluate with reagent RBCs. Were the DAT positive because of a warm autoantibody, strongly positive reactions (2+ to 4+) would be obtained when testing the eluate.

In a recent report summarizing the experience of this author's laboratory, more than half of the cases of drug-induced immune hemolytic anemia (DIIHA) investigated demonstrated reactivity in initial antibody detection tests.[2] Serologic results in this case could easily be misconstrued as a warm autoantibody. Positive reactivity without adding drug to the test may have two explanations. If the patient is on the drug at the time of testing, the drug is likely circulating in the patient's plasma. Alternatively, a drug-independent warm autoantibody may be present. It is possible that drug-independent autoantibody reactivity may resemble reactivity seen when a patient experiences a delayed transfusion reaction and is producing not only

Table 4. Results of testing patient's serum with and without the presence of drugs

Serum	PBS			Zosyn			Piperacillin			Tazobactam		
	RT	37°C	IAT	RT	37°C	IAT	RT	37°C	IAT	RT	37°C	IAT
Patient	0*	0	2/11†	8/24	8/22	8/17	4/18	4/18	4/9	0	0	1/8
Normal	0	0	0	0	0	0	0	0	0	0	0	0
Positive control	NT	NT	NT	3+	3+	4+	3+	3+	3+	0	0	0
Eluate	NT	NT	NT	NT	NT	3+	NT	NT	2+	NT	NT	NT

RT = room temperature, NT = not tested

* agglutination strength (0 – 4+)

† titer/score

alloantibody but also autoantibody. A careful drug history is imperative when the eluate is negative.

This patient was receiving Zosyn (Wyeth Pharmaceuticals, Philadelphia, PA) 2 g intravenously every 6 hours. Zosyn is a broad-spectrum antibiotic consisting of piperacillin sodium in combination with the β-lactamase inhibitor tazobactam sodium. Arndt et al.[3] and Johnson et al.[4] reported patients with DIIHA caused primarily by piperacillin antibodies. Johnson et al.[5] later reported four cases associated with Zosyn. Drug-dependent antibody was detected in the presence of both Zosyn and piperacillin; however, reactivity was greater with Zosyn.

Drug studies were performed testing the patient's serum in the presence of the drugs Zosyn, piperacillin, and tazobactam because recent reports have shown that Zosyn- and piperacillin-dependent antibodies react best in this method.[3-6] The results are in Table 4.

Although there are weakly positive reactions with the patient's serum and PBS, reactivity is significantly increased in the presence of both Zosyn and piperacillin, consistent with drug-dependent antibodies. In addition, the eluate was positive in the presence of both Zosyn and piperacillin.

Finally, autologous adsorptions removed the antibody reactivity and no underlying alloantibodies were detected in the adsorbed serum.

Conclusions

Zosyn- and piperacillin-dependent antibodies were detected in the patient's serum. Had all testing for drug-dependent antibodies been negative, repeating the tests using enzyme- (ficin or papain) treated reagent RBCs may have enhanced the reaction with the drug-dependent antibody. Had testing continued to yield negative results, it may have been beneficial to treat RBCs with each drug and then test the drug-coated RBCs. If there is clear evidence of hemolysis as in this case, testing by several methods may be required to detect a drug-dependent antibody. As with common RBC alloantibodies, drug-dependent antibodies do not "read the book." One can never be certain when the next type of drug-dependent antibody may be identified!

The use of Zosyn was discontinued, and the patient's hemoglobin stabilized after two units of LRBCs were administered. The patient was informed that it is important to avoid Zosyn and piperacillin in the future to prevent a repeated hemolytic event perhaps worse than this episode.

In summary, this is a case of DIIHA caused by Zosyn- and piperacillin-dependent antibodies. Initial serologic results were identical to those seen in cases of WAIHA. The negative eluate was critical in differentiating this case from WAIHA, emphasizing the importance of performing an eluate on initial workup of apparent cases of WAIHA. This also demonstrates the importance of a careful drug history in the face of significant RBC hemolysis and serologic evidence of WAIHA.

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