

Case Report

Auto-Immune Hemolytic Anemia in Patient who his Serum React with all ABO Blood Group

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ABSTRACT

There are several irregular antibodies associated with various blood group systems which may cause some problems during blood cross matching in transfusion.

The atypical antibodies are included auto and alloantibodies such as anti-I, anti-HI, anti-P... . In order to detect these antibodies, generally the agglutination reaction technique and anti-human-globulin (coombs) tests would be performed and a panel of identified red blood cells will use if necessary for further investigation.

During our work, we encountered with one serum sample that showed agglutination reaction with all the blood groups (A, B, O, and AB).

We tested pooled red blood cells with OI group of adult and pooled cord red blood cells of Oi group with the patient serum. it was shown that the serum was reactive with OI but not with Oi. For confirmation of the result, the sample was sent to Institute of Immunohematology (I.I.H.), India. The report approved that the serum contained anti-I specificity. To solve the transfusion problem for this patient, the recommendation is using the blood group with minimum coombs titration if the patient life is in threatened. Further investigations disclosed that the patient had leukemia.

Key Words: Anti-I, Ii antigen, Allo-Autoantibody.

The atypical antibodies are categorized as: (a) allo-antibodies which are produced, as a rule, when the corresponding antigen is absent, and b) auto antibodies which are directed towards the self-antigens. Allo-antibodies are further divided into naturally occurring and immune antibodies. The later developed because of stimulation by specific blood group antigens through blood transfusion or pregnancies. Many of the auto-antibodies show the blood group specificities mainly within the I-i and the Rh systems¹.

Giblett and crookston (1964) reported that red cells of thalassaemia major (not recently transfused) reacted almost as strongly with anti-i as did the cells of newborn infants Oi². Differential observation on the red cell I antigen was thought to be due to heterogeneity existing in different anti-I antibodies³. Cord red cells react weakly with anti-I and strongly with anti-i. During the first 18 months of life the red

cells gradually come to react strongly with anti-I and weakly with anti-i. This phenotype (strong I, Weak i) is retained by healthy persons throughout the life⁴.

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Anti-I is found as an alloagglutinin, acting best at 4°C (titer 16-32) and sometimes at room temperature, in the serum of most i subsets⁵.

Numbers of studies have indicated increased Ii content in leukemia and other hematologic malignancies^{6,7,9,10}. It has been observed that red cell ABH antigens are weakened and I antigen increased in leukemia giving problems in cell grouping. Similar problems may be experienced while typing the red cells of newborn infants^{7,11,13}.

From anti-HI, AI, BI, Hi, Bi, P₁I, P₁I^T, and HILe^b, list of antibodies which react only with red cells carrying I or i and a second antigen, anti-HI is the

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commonest. Antibodies with this specificity often cause problem when pre-transfusion tests are carried out at room temperature. It is recommended that such antibodies are first neutralized by adding pooled human sera before performing the cross matching^{8,12}.

During the routine works in st. Al-zahra hospital blood banking in spring and summer 2003, among the sera of patients, there was one case which reacted with all ABO blood groups of RBCs. The serum was taken from a 49 years old man with B+ blood group in recorded file, but with sever anemia in present time of typing who need blood transfusion. It was too much difficult to find a compatible blood group for him and it was vital to find a blood having at least lower incompatibility. We were using serological technique and cross matching to find out the lowest grade of incompatible blood for transfusion. So, when the incompatibility in blood transfusion occurred, the next steps would be the consideration of the sort of auto-allo-antibodies with the following procedures. In Our case we tried to identify the reactive antibody, and through our effort, the anti-I antibody

was detected and approved in Institute of Immunohematology (I.I.H), India.

In order to clear the type of antibody in his serum, the following method according to American Association of Blood Bank (AABB 1999) was used:

- 1) Serial two-fold dilutions of the serum in saline prepared. The dilution ranges were from 1 in 2 to 2096 (12 tubes) and the volumes prepared were less than 1 ml.
- 2) 3 drops of each dilution with 1 drop of 5% saline suspension of each test RBCs sample were perfectly mixed.
- 3) All samples were incubated at room temperature for 15 minutes. Then samples were centrifuged and the RBCs examined macroscopically for agglutination, graded, and the results were recorded.
- 4) The tubes transferred to 4°C and incubated at this temperature for 1 hour after centrifugation. the RBCs were examined for agglutination.
- 5) The titration of antibody scored according to agglutination reaction from 4+ to weak (w+).

Table 1. the titration of antibody (anti-I) which was tested in different temperatures

Titration of	Antibody in patient Serum		Normal Serum control
	Antibody	Titration	
0-4	OI	1/512	0-1/8
20-22	OI	1/64	0
37	OI	1/16	0

Table2. Comparative Reactions with O -/- blood group to identify the specificity of unknown serum in Cold-Reactive condition

Red cells	Patient serum	Antibody Specificity				
		Anti-I	Anti-i	Anti-H*	Anti-IH*	Anti-Pr
Oi(adult)	0	0	+	≡	0	≡
Oi(cord)	0	0	+	<	0	≡
A ₁ I	≡	≡	≡	0	0	≡
OI	+	+	-	+	+	0
OI(enzyme-treated)	+	+	<	+	+	0
Autologous	≡	≡	≡	0	0	≡

0 : non reactive, ≡ : equal to OI red cells, < : equal to or weaker than OI red cells, + : reactive

* Anti-H and anti-IH antibodies are seen predominantly in A1 and A1B individuals, however, it is clear that O RBCs contain maximum H antigen in comparison with other blood groups, therefore shows strongest reaction with anti-H and anti-IH.

The titer of antibody in the patient serum in different temperatures (0-4, 20-22, 37°C) with OI RBCs are shown in table 1. These results showed that the antibody in question should be in the category of cold auto antibodies.

The case showed anti-I specificity, which reacted with OI adult cells and had no reaction with Oi cord cells. In addition, to demonstrate the specificity of cold-reactive auto antibody (anti-I) in patient serum, we used various RBCs with different types of antibodies as shown in table 2.

Discussion

The serum with atypical antibody was tested with a panel of red blood cells, containing known antigens. depending on the reactivity with the different panel of red cells, the antibody specificity is uncertain with serological techniques. In more complicated cases the tests with rare blood types, including "minus-minus" phenotype may help to determine the specificity of antibody¹. Alteration of the Ii antigen has been a matter of conflicting reports. In patients with a variety of hematological disorders, i activity of the red cells may increase again, usually without a demonstrable decrease in I activity. This is particularly true in situations of marrow stress (Giblett et al, 1964), such as what occurs in thalassemia major or chronic hemolytic anemia, hypoplastic anemia, as well as leukemia^{2,7}. Anti-I is

a fairly common autoantibody if tests are performed at 4°C. On occasion, this IgM autoantibody, may be identified if room temperature testing is performed. This antibody reacts with all (or nearly all) adult red cells but fails to react with cord red cells^{3,4}. Also there was a spectrum from the sera which clearly had anti-i specificity to those which were clearly anti-I, with many between them which reacted more strongly with cord cells than with adult cells or vice versa⁵. Anti-I is an IgM autoantibody reacts most strongly with cord red cells, less strongly with I-adult cells, and most weakly with i adult red cells⁶. In our series, only one patient showed auto-immune hemolytic anemia with autoantibody reacting at different temperatures (0-4°C, 20-22°C, 37°C) and showed the presence of auto anti-I specificity with 1/512 titer at 0-4°C. Such cases have been reported from different parts of the world^{7,9,12,13}. However, caring and managing these cases need a suit background knowledge of transfusion problems, for both medical technologist in laboratory and physician to consider all aspects of hemolytic anemia^{12,13}. With further seek and considering the patient's hospital record it was known that the patient had leukemia (CMI) in the time of transfusion, So in our case we could speculate that this auto-immune syndrome may have occurred secondary to blood malignancy.

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