Studies on Causes Inducing Positive Reaction in Self-Controls of Coombs Tests Carried Out as Cross-Matchings and on Transfusion Results in These Cases

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Abstract

Self-control of indirect Coombs tests gave positive results at the frequency of 5.5% (585 cases) among 10,727 pretransfusion cross-matchings. Drugs (antibiotics, methyldopa) (46.3%), adsorption of complement along with anti-I (17.8%), autoantibodies (3.6%), and anti-A or anti-B (0.7%) were the main causes of these positives. On the other hand, no such causes could be found in the remaining 30.8%.

In the 460 cases who received blood transfusion in spite of their positive self-controls, no clinical side effects were observed.

Introduction

Since some media, such as albumin or LISS (low-ionic-strength solution), have been introduced into the Coombs test for pretransfusion cross-matching in order to elevate the sensitivity of the test for the detection of anti-red-blood-cell (RBC) antibodies¹⁾, self-control in the Coombs test, using the patient's RBC and their own sera, often gives positive results due to causes other than cold-reactive anti-I, warm-reactive anti-RBC autoantibodies, rouleau formation, or pan-agglutination. Among the probable causes may be anti-RBC antibodies derived from plasma preparation products which had been transfused²⁾, non-specific adsorption of complement along with cold-reactive anti-I antibody or that of plasma proteins to RBC membrane protein³⁾.

Reported here, are the results of studies on the causes inducing self-control positive results in the Coombs test, and also on the side effects of blood transfusions in these cases.

Materials and Methods

Pretransfusion cross-matchings had been carried out in 10,727 cases during the last three

years, by means of the saline, albumin, Coombs, and bromelin methods. When both the self-control application, using the patient's RBC and his sera, and the accessory application, using the patient's RBC and donor's sera, gave positive results in the indirect Coombs test (i.C.), the direct Coombs test (d.C.) was performed, and the immunoglobulin class of the antibody was also determined using monospecific antiglobulin or anticomplement sera. Moreover, eluates from the RBC membrane obtained by Landsteiner's heat method⁴⁾, the chloroform or trichloroethylene methods⁵⁾ were examined in order to determine the specificity of the antibody. When the eluate showed no antibody activity, we examined whether or not the patient had received drugs, particularly drugs such as methyldopa, cepheids (CEPs), penicillins (PCs), or plasma preparation products during the previous month. Cases who had been administered CEPs or PCs at over three grams per day for more than seven days were also included in this drug-induced classification. Elution tests were also carried out in cases showing d.C. negative despite their showing self-control positive.

Four hundred and sixty patients among them, who received blood transfusion in spite of the positive control tests, were studied as to side effects following the blood transfusion. For identification of the detected antibodies, three commercialized RBC panels (Ortho Diagnostic Systems Inc., U.S.A., American Dade, U.S.A., and Gamma Biologicals Inc., U.S.A.) and our self-made panel cells were used.

Results

Five hundred and eighty-five of the 10,727 cases (5.5%) gave positive results in the self-control i.C. Among them, 218 cases (37.3%) also showed a positive d.C. while the remaining 367 cases (62.7%) were negative. The majority of these 218 d.C. positive cases, i.e. 134 (61.5%), gave positive results against both polyspecific anti-human serum (p.s.) and anti-IgG serum, followed by 58 cases (26.6%) who were positive against both p.s. and anti-C3, the remaining 26 cases (11.9%) being positive against p.s., anti-IgG, and anti-C3 serum. Thus, over 70% of them were positive against anti-IgG serum. In 271 of the 585 cases (46.3%), some drugs seemed to lead to a self-control positive i.C. On the other hand, in 9 (1.5%) of the cases, methyldopa had been administered for more than several years, and IgG anti-RBC autoantibodies could be detected in their eluates. In most of the other cases, antibiotics, particularly CEPs (60.7%) or PCs (10.3%), had been administered individually or in combination (28.6%), but no anti-RBC autoantibody could be detected in these eluates. The second important cause of the self-control i.C. positives was cold-reactive anti-I antibody or adsorption of complement along with anti-I, which was seen in 104 cases (17.8%). Furthermore, warm-reactive anti-RBC autoantibody was found to be the cause in 21 cases

HDN: hemolytic disease of the newborn

Fig. Possible causes inducing self-control positives in the Coombs test

Table. Characteristics of detected antibodies and results of blood transfusion in 460 cases showing self-control positives

Kind of antibody	Specificity on blood type	Globulin class of antibody	No. of cases	Characteristics of detected antibody		Transfused RBC	Side effect
				serum	eluate		
Anti-RBC auto-antibody	(+)	IgG	1	Anti-C	Anti-C	ccDEe	(-)
			1	Anti-M+C+e	Anti-C+e	NN, ccDEE	(-)
		IgM + IgG + C3	1	Anti-N	Anti-N	MM	(-)
	(-)	IgG	4	all panel cells (+)	all panel cells (+)	washed RBC or red cell concentrate with compatible ABO & Rh type	(-)
		IgG + C3	3				
Due to drugs	(-)	IgG	2	„ (methylidopa)	„		washed RBC or red cell concentrate with compatible ABO & Rh type
		IgG + C3	134	all panel cells (-)	all panel cells (-)	(-)	
		IgM + IgG + C3 + C3d	99	(PCs, CEPs)		(-)	
Anti-A, -B derived from plasma preparation	(+)	IgG	2	Anti-A	Anti-A	washed RBC of O type	(-)
			2	(-)	Anti-A or B	washed RBC or red cell concentrate with compatible ABO & Rh type	(-)
Cold-reactive autoantibody	(+)	IgM + IgG + C3 + C4	2	Anti-I	Anti-I	washed RBC or red cell concentrate with compatible ABO & Rh type	(-)
		IgM + C3 + C4	106	Anti-I	Anti-I or (-)		RBC with compatible ABO & Rh type
		C3 + C4					
		C3 or C4					
Undetermined (possibly PCs, CEPs or Anti-A, B)	(-)	IgA	103	(-)	(-)	RBC with compatible ABO & Rh type	(-)
		IgM					
		IgG					
		C3					
		C4					

PCs : penicillins, CEPs : cepheims

(3.6%), and hemolytic disease of the newborn (HDN) was found in 4 cases (0.7%). Anti-A or anti-B antibodies originating from plasma preparation products were found in 4 cases (0.7%), and sepsis in 1 case (0.2%). In the remaining 180 cases (30.8%), no probable causes could be pointed out (Figure).

Although blood transfusions were dared in 460 of the 585 cases showing self-control i.C. positive, no side effects were clinically detectable in any of these cases (Table).

Discussion

Although the self-control test using the patient's sera and their own RBC is often very useful for analysis of positive results in cross-matchings, it is not always carried out, as it is not always recommended, particularly in pretransfusion cross-matchings^{6, 7, 8)}.

Whenever the self-control test gives a positive result, we should decide how to deal with it as soon as possible, especially in deciding whether to dare or to postpone a blood transfusion.

In this study, the frequency of the positive self-controls was 5.5%. Studies on the causes of the positive reactions revealed that about a half (46.3%) of the cases were induced by drugs. Methyldopa is known to produce anti-RBC autoantibody, and the cepheims to alter the RBC membrane proteins so as to result in their non-specific adsorption of plasma proteins, and the penicillins to act as haptens bringing about the production of antibodies combining with the drug-RBC complex. It has been reported that 3 to 5% or 40 to 75% of patients who had been administered cepheims gave positive results in the d.C.⁸⁻¹⁴⁾. Moreover, the causative presence of specific anti-penicillin¹⁵⁻¹⁷⁾ or of anti-cephem antibodies^{11, 13, 14)} has also been suggested. And most of our drug-induced positives in the self-control test were considered attributable to antibiotics such as the cepheims or penicillins, although the presence of drug-specific antibodies could not yet be clarified.

Since complement adsorption to the RBC membrane along with cold-reactive antibodies, particularly anti-I antibody, was the second main cause, the test tubes have to be handled speedily and in warm status, to avoid cooling.

As we have reported earlier, commercial heat-treated human albumin or plasma preparation products can contain anti-A and/or anti-B antibodies of the IgG type at high titers²⁾. Here too, IgG anti-A or anti-B antibodies, probably originating from transfused plasma preparation products, could be detected in eluates from the patients' RBC in 4 cases.

However, in the remaining 30.8% of the cases showing self-control positive in pretransfusion cross-matching, the cause could not be clarified. Many patients who had been administered with antibiotics or plasma preparation products were found here, too. In these cases, the amount of anti-A or anti-B antibody transferred by the plasma preparation products or of

proteins adsorbed to the RBC membrane might be too small to induce a positive Coombs test.

As mentioned above, the administration of drugs, particularly antibiotics and plasma preparation products, seemed to be the main cause of the positive control test. We have to do our best to get thorough clinical information, particularly as to therapies previously received, and to interpret the obtained results of the tests on the basis of this information. On the other hand, the introduction of media, such as LISS or albumin, which enhance the ability of the test in detecting antibodies, may well have elevated the rate of the positive results.

In spite of the positive results of the self-control tests, blood transfusion could be done successfully in 460 cases. Although washed red blood cells were usually used, red cell concentrates were transfused to some of these cases, due to urgency or other reasons, without any side effects.

The self-control test using the patients' RBC and their own sera should be set up, whenever the direct or indirect Coombs test is carried out for pretransfusion cross-matchings. And, when these self-controls give positive reactions, we should consider the above-mentioned causes.

Conclusion

The self-control applications of the indirect Coombs test, using the patients' RBC and their own sera, gave positive results at the frequency of 5.5% among 10,727 pretransfusion cross-matchings carried out in our blood transfusion unit during the past three years.

Drugs such as the cepheims or methyldopa, the adsorption of complement to the RBC membrane along with cold-reactive anti-I antibody, warm-reactive autoantibodies, and anti-A and/or anti-B antibodies originating from plasma preparation products which had been administered were the main causes of positive findings. On the other hand, no such causes could be found in the remaining 30.8% of the positive cases. Both the recent improvement of the media to enhance the ability of the test in detecting antibodies and the recent increment in the usage of antibiotics or plasma preparation products might be the synergistic cause of the frequent encounters with positive self-control results.

In 460 cases who received blood transfusion in spite of their positive self-control findings, no clinical side effects were observed.

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