Antibody elutions in Thai patients with a positive direct antiglobulin test

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Background. The direct antiglobulin test is performed to determine whether an anaemic patient with evidence of haemolysis has autoimmune or alloimmune haemolytic anaemia.

Materials and methods. We determined the antibody specificity of eluted IgG antibodies from patients' blood samples with a positive direct antiglobulin test. Overall, 134 Thai patients were included in this study. EDTA blood samples were obtained from recently transfused patients, patients with unexplained anaemia and patients who had serum antibodies detected during routine pre-transfusion tests from different hospital blood banks. These complicated samples were sent to the National Blood Centre of the Thai Red Cross Society for investigation and to find compatible blood components. Each blood sample underwent a direct antiglobulin test with the gel technique using polyspecific antihuman globulin and mononospecific anti-IgG and anti-C3d. Acid eluates were prepared from the samples for which the direct antiglobulin test was positive and the specificities of the eluted antibodies were determined by the gel technique.

Results. Of the samples tested, 101 showed a positive direct antiglobulin test result (75.4%) using polyspecific antihuman globulin sera whereas only 95 samples (70.9%) were positive with anti-IgG or anti-IgG and anti-C3d. Moreover, 54 of 95 eluates (56.8%) were positive for antibody screening and tested with the reagent panel cells. Twenty-one eluates had specific alloantibodies, which were concordant with the findings in the patients' sera and all patients had a history of blood transfusion. Additionally, 33 eluates contained pan-agglutinins. Interestingly, alloantibodies could be determined using titration studies in 5 of 26 eluates with pan-agglutinins.

Conclusion. Although the direct antiglobulin test is not routinely performed in pre-transfusion screening, this test and elution studies would be useful in patients with a history of previous transfusions, and in those for whom compatible blood cannot be found.

Key words: eluate, positive direct antiglobulin test, Thai patients.

Introduction

The direct antiglobulin test (DAT) is performed to determine whether an anaemic patient with evidence of haemolysis has an autoimmune or alloimmune haemolytic anaemia. A positive DAT with anti-immunoglobulin G (IgG) indicates that IgG antibodies are bound to the red blood cells (RBC). These antibodies can be eluted from the RBC and their specificity can be determined. Hence, in order to demonstrate that the recipient has become sensitised to an alloantigen on transfused RBC, the antibodies in the eluate must be identified. If, on the other hand, the antibody agglutinates all reagent RBC, the patient has probably produced an autoantibody[1,2].

In general, approximately 1% to 3.5% of unselected hospital patients will have a positive DAT, which may or may not be associated with haemolysis[3-6]. However, it has been reported that the incidence of

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positive DAT is significantly increased in patients with haemolytic transfusion reaction, autoimmune diseases, congenital and acquired immunodeficiency syndromes and malignancy[1]. It has been suggested that flow cytometry and antibody detection in the eluate of post-transfusion sample can be used to reveal minor populations of IgG-coated RBC in cases of suspected haemolytic transfusion reaction[7]. A previous study for an external quality assessment programme in blood group serology in Thailand found that about 15% to 30% of blood bank laboratories participating in this programme did not routinely perform antibody identification and that they usually sent the blood samples with positive antibody screening results to the National Blood Centre, Thai Red Cross Society for investigation and crossmatching[8]. A more recent study reported that the prevalence of positive DAT in patients' samples obtained from these blood bank laboratories was high (65.8%) and strong DAT positive results were found in patients with autoimmune diseases and in patients who had received blood transfusions within the preceding 3 months. However, the specificities of the antibodies on the RBC were not determined[9]. The purpose of the current study was to determine the specificities of IgG antibodies from the eluates of patients' blood samples with positive DAT and also to assess how frequently eluates contributed novel serological information for the blood bank laboratories.

Materials and methods

Subjects

One hundred and thirty-four EDTA blood samples were obtained from patients who were anaemic, for reasons other than blood loss, had been recently transfused, or had serum antibodies detected during routine pre-transfusion tests from different hospital blood banks in Thailand. These complicated samples were sent to the National Blood Centre of the Thai Red Cross Society Bangkok, Thailand between September 2009 and February 2010 for investigation and to find compatible blood components. Each blood sample underwent a DAT using the gel technique at the Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumtani, Thailand. To increase the validity and reliability of the evaluations, laboratory technicians performing DAT were blinded to the antibody specificity results in each patient's serum, which was routinely performed at the National Blood Centre of the Thai Red Cross Society.

Methods

Each sample underwent a DAT with a polyspecific antihuman globulin reagent (anti-IgG and anti-C3d) using the gel technique with a LISS/Coombs card (DiaMed, Cressier sur Morat, Switzerland). Briefly, 50 mL of 0.8% RBC suspension in low-ionic strength solution were added to the reaction chamber of the ID card. The ID card was centrifuged at 90 x *g* in a designated centrifuge (ID-Centrifuge 12 SII, DiaMed, Cressier sur Morat, Switzerland) for 10 min. The DAT reactions were graded as 4+, 3+, 2+, 1+, w+ and negative, as indicated by the manufacturer.

If the DAT was positive, a monospecific anti-IgG and anti-C3d (DC-Screening II) card (DiaMed, Cressier sur Morat, Switzerland) was subsequently employed. Elutions were performed using the rapid acid elution kit (DiaCidel, DiaMed, Cressier sur Morat, Switzerland) and the antibody specificity was determined with an indirect antiglobulin test using the gel technique with the same reagent screening cells and panel cells used for testing the serum (National Blood Centre, Thai Red Cross Society, Bangkok, Thailand and DiaMed, Cressier sur Morat, Switzerland). Alternatively, titration studies in the eluates demonstrating pan-agglutination, which were reactive with all reagent RBC, were implemented in order to identify alloantibody specificities[1,10].

Results

A total of 134 blood samples underwent a DAT using the gel technique and in 101 (75.4%) samples the test was positive. Only 95 blood samples (70.9%) reacted with anti-IgG (n=51) or anti-IgG and anti-C3d (n=44), whereas the other six blood samples reacted with only anti-C3d. The strengths of the DAT positive reactions of these 95 blood samples were w+ (12.6%), 1+ (31.6%), 2+ (32.6%), 3+ (16.3%) and 4+ (6.3%), as shown in Table I. The history of blood transfusions in these patients was also determined revealing that 65 patients (68.4%) had received blood transfusions within the preceding 3 months, 22 patients (23.2%) had a history of blood transfusions more than 3 months before the testing and 8 patients (8.4%) had not received blood transfusions. Fifty-four

Table I - Characteristics of the strengths of DAT reactions and the eluates in 95 blood samples

	Characteristics of the eluates							
DAT strength	Alloantibodies N=21	%	Pan-agglutinins N=33	%	Non-reactive N=41	%	Total N=95	%
W+	4	19.0	0	0	8	19.5	12	12.6
1+	11	52.4	3	9.1	16	39.0	30	31.6
2+	6	28.6	14	42.4	11	26.8	31	32.6
3+	0	0	12	36.4	4	9.8	16	16.3
4+	0	0	4	12.1	2	4.9	6	6.3

Table II - Antibody specificities found in the eluates compared with those in patients' sera (N=21)

N.	ID N.	Antibody specificity in Eluate	Serum
1	E6	Anti-D	Anti-D, Unidentified
2	E10	Anti-E	Anti-E, Anti-c, Anti-Mi^a
3	E51	Anti-E	Anti-E, Autoantibody
4	E92	Anti-E	Anti-E, Anti-c, Anti-Mi^a
5	E20	Anti-C	Anti-C, Anti-e, Anti-Mi^a
6	E78	Anti-Jk^a	Anti-Jk^a , Anti-E, Anti-c, Autoantibody
7	E102	Anti-Jk^a	Anti-Jk^a
8	E19	Anti-C, Anti-Jk^a	Anti-C, Anti-Jk^a , Anti-e, Anti-P1, Anti-Mi^a
9	E44	Anti-E, Anti-Jk^a	Anti-E, Anti-Jk^a, Anti-Mi^a
10	E16	Anti-E, Unidentified	Anti-E, Anti-c, Anti-Mi^a, Unidentified
11	E33	Anti-E, Unidentified	Anti-E, Anti-P1, Autoantibody
12	E131	Anti-E, Unidentified	Anti-E, Anti-Mi^a, Unidentified
13	E25	Anti-e, Unidentified	Anti-e, Anti-C, Anti-Jk^a, Autoantibody
14	E38	Anti-Mi^a, Unidentified	Anti-Mi^a, Anti-Jk^a
15	E27	Anti-Jk^a, Unidentified	Anti-Jk^a, Anti-E, Anti-c, Anti-Mi^a, Unidentified
16	E12	Anti-Jk^b, Unidentified	Anti-Jk^b, Anti-E, Unidentified
17	E22	Anti-E, Unidentified	Anti-E, Anti-Mi^a, Autoantibody
18	E18	Anti-Mi^a, Unidentified	Anti-Mi^a, Anti-C, Anti-e, Autoantibody
19	E103	Anti-Mi^a, Unidentified	Anti-Mi^a, Unidentified
20	E14	Unidentified	Anti-E, Anti-c, Anti-Mi^a
21	E126	Unidentified	Anti-P1, Anti-Fy^b, Anti-Mi^a, Unidentified

of 95 eluates (56.8%) were positive for antibody screening and only these 54 eluates were then tested with the reagent panel cells. The antibody specificity was demonstrated in 21 eluates (22.1%), and the antibodies present in the eluates were also detected in these patients' sera by routine pre-transfusion tests, as shown in Table II. All of these 21 patients had received blood transfusions and the strengths of the DAT positive reactions ranged from w+ to 2+ (Table I). On the other hand, the DAT strengths of the other 33 samples (34.7%) ranged from 1+ to 4+ (Table I) and all eluates demonstrated pan-agglutination (i.e. they agglutinated all reagent RBC and cord blood). Twenty-six of these 33 eluates were diluted with 0.85% normal saline and tested with the reagent RBC to determine the specificity of the alloantibodies. Interestingly, the antibody specificity was identified in five diluted

eluates, while antibody specificity could not be identified in two serum samples (n. 84 and n. 136), as shown in Table III.

Table III - Type of antibodies recovered in the diluted eluates compared with those in patients' sera (N=5)

Eluate N.	Types of antibodies in Eluate		Serum
	Undiluted	Diluted	
E37	Pan-agglutinins	Anti-C, e, Unidentified	Anti-C, e, Autoantibody
E42	Pan-agglutinins	Anti-E, Unidentified	Anti-E, Mi^a, Jk^a, Fy^a
E88	Pan-agglutinins	Anti-C, e, Unidentified	Anti-C, e
E84	Pan-agglutinins	Anti-E, Jk^a, c	Unidentified
E136	Pan-agglutinins	Anti-Jk^b, Unidentified	Unidentified

Discussion

The DAT is widely used because it is simple, quick and inexpensive. It should be performed when the presence of haemolysis has been established and is one of the most important diagnostic tests for determining whether haemolytic anaemia is of autoimmune and/or alloimmune nature. If the DAT is performed when immune-mediated haemolysis is suspected, it has a good predictive value[11]. Moreover, elutions can be performed as part of a serological investigation of a haemolytic transfusion reaction or when ordered by a clinician for the diagnosis of immune-mediated haemolysis.

In blood bank laboratories, the gel technique can be used for both antibody detection and the DAT because it is simple and easier to read the reactions and the results are more likely to be accurate than those of the conventional tube technique[12-17]. In this study, the DAT was performed, using the gel technique, on blood samples from 134 patients. Most of the patients who had IgG antibodies or IgG and C3d coated on their RBC (91.6%) had a history of blood transfusions, confirming results of a previous study[9]. In addition, strong DAT positive results ($\geq$ 2+) were found more commonly in patients with pan-agglutinins than in patients who had had previous transfusions and had specific alloantibodies. However, 54 eluates (56.8%) were positive for antibody screening and 41 eluates (43.2%) were negative for antibody screening. Regarding previous studies of patients with a positive DAT, it was found between 66.6% and 68.3% of these patients had non-reactive eluates. It is, therefore, recommended that the DAT should be included in pre-transfusion testing in order to detect alloantibody formation promptly before such antibodies are present in the serum, especially in patients with a history of previous blood transfusions[18,19].

Although the results of antibody specificities in the eluates were concordant with those in the patients' sera, the application of special techniques such as adsorption and antibody titration is useful. Adsorption is used to remove serum autoantibodies and enable the subsequent detection of the underlying alloantibody in patients with autoimmune haemolytic anaemia who have received blood transfusions more than 3 months previously. However, it is not suitable for use in recently transfused or severely anaemic patients. In these latter cases, alloadsorption is essential, but it has the disadvantage of adsorbing alloantibodies against high prevalence antigens. Moreover, antibody titration is useful in order to solve complex antibody problems[1]. In this study, it was found that the antibody specificity could not be determined in two patients' serum samples or in the undiluted eluates, but these antibodies (anti-E, anti-Jk^a and anti-c in eluate n. 84 and anti-Jk^b in eluate n. 136) were identified in diluted eluates. Importantly, these antibodies are commonly found among the Thai population[20,21]. Group- and type-specific compatible red cells are needed for these patients with alloantibodies.

In conclusion, the DAT is not routinely performed in pre-transfusion testing; however, the DAT and additional tests such as adsorption-elution and eluate antibody titration would be beneficial for patients who have recently received transfusions for whom compatible blood cannot be found.

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