Frequencies and specificities of red cell alloantibodies in the Southern Thai population

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Abstract:

Context: Detailed reports of red cell alloantibody frequencies and specificities in the Thai population are limited. The aims of this study were to determine the specificity and compare the frequency of alloantibodies detected using column agglutination technology (CAT) and conventional tube techniques in blood donors and previously transfused patients.

Settings and Design: We retrospectively reviewed antibody screening and identification records for two time periods: January-December 2006 during which conventional tube techniques were used and January 2008-December 2009 when CAT was used. Results: The overall prevalence of alloantibodies in both patients and donors when using conventional tube techniques was 0.7%, for patients only was 0.9% and donors 0.6%. The most frequent antibodies detected in both groups were anti-Le a, anti-Mí b, anti-Le b, anti-P, and anti-E. When using CAT, alloantibodies were found in 0.8% of patients and 0.13% of donors with the five most common alloantibodies found in patients were anti-Mí a, anti-E, anti-Le b, anti-c and anti-Le b respectively. Similarly the common alloantibody specificities in donors were anti-Le a, anti-Mí b, anti-Le b, anti-M and anti-D. Conclusions: One of the most commonly identified alloantibodies in the Thai population studied was anti-Mí a suggesting that Mí a positive red cells should routinely be included in antibody screening and identification in this population. For antibody screening and identification, CAT method detected immune and warm alloantibody (ies) more frequently than that associated with conventional tube techniques.

Key words: Alloantibody frequency in thais, antibody identification, antibody screen, column agglutination technology, conventional tube method

Introduction

Sensitization to red cell antigens may result from previous transfusions, pregnancy, transplantation or injection of immunogenic material. Blood group antibodies may also be naturally occurring. The frequency of alloantibodies varies depending on population demographics and the sensitivity of detection techniques used.

The southern Thai population have different ethnic origins compared to other regions of Thailand. The majority of the southern population is local Thai living in the upper South. Thai people living in lower southern Thailand near the border with Malaysia often may have Malay ancestry.

The aims of this study were to determine the specificity and compare the frequency of alloantibodies detected using column agglutination technology (CAT) and conventional tube techniques using normal saline suspended red cells in blood donors and previously transfused patients.

Settings and Design

Antibody screening and identification

All patient’s blood group, antibody screen, antibody identification and cross-match records from the Blood Bank and Transfusion Medicine Unit, Songklanagarind University Hospital for the 1 year period of the 2006 January-December period and the 2 year period of January 2008-December 2009 were reviewed. Similarly blood donor laboratory records from January 2008-December 2009 were reviewed.

Prior to 2007 conventional tube techniques using red cells suspended in normal saline were routinely used for antibody screening using two group O screening cells. Antigen coverage included D, C, E, c, e, Fy a, Fy b, Jk a, Jk b, Le a, Le b, Mí a, Mí b, M, N, K, k, S, s, P1, Lu a, Lu b and Dr a.

Antibody screening and antibody identification panel cells were provided by the Thai National Blood Centre (NBC) of Thai Red Cross.
The indirect antiglobulin tube technique was used for antibody screening. Antibody identification techniques included a room temperature incubation phase, a 37°C phase and indirect antiglobulin phase using polyspecific anti-human globulin (containing anti-IgM, anti-IgG and anti-C3d which was manufactured by the Thai NBC).

Column agglutination technology was introduced into routine laboratory techniques for ABO and RhD grouping, antibody screening and antibody identification in 2008. All blood grouping and antibody screening was performed using an automated platform (AutoVue Innova™, Ortho Clinical Diagnostics, USA). ABO and RhD groups were tested using BioVue ABO-Rh/Reverse Grouping cassettes (Ortho BioVue® System, Ortho Clinical Diagnostics, USA). Standard reverse grouping cells were A, and B cells (0.8% Affirmagen®, Ortho-Clinical Diagnostics, USA).

Antibody screening was performed using BioVue Poly cassettes (Ortho BioVue® System). Three group O screening cells were used in the antibody screen. Two screening cells were obtained from Ortho Clinical Diagnostic (0.8% Selectogen®, Ortho-Clinical Diagnostics, USA); Antigens covered included: D, C, E, c, e, Fy a, Fy b, Kp a, Kp b, and Js. In addition a third group O screening cell which was Mr and Dr positive, was provided by the Thai NBC. 0.8% cell suspensions of this screening cell were made in low ionic strength salt solution (LISS) supplied by Ortho Clinical Diagnostics (Ortho® 0.8% red cell diluent).

Antibodies were further identified by manual CAT techniques using either DiaMed ID LISS-Coombs micro typing cards (DiaMed, Switzerland) or BioVue Poly cassettes (Ortho BioVue® System, Ortho Clinical Diagnostics, USA) using a panel of eleven group O red cells provided by the Thai NBC. The CAT indirect antiglobulin technique was employed and performed at 37°C according to the manufacturer’s instructions. Selective red cell antigen typing for the corresponding blood group alloantibody(ies) was performed following antibody(ies) identification.

**Results**

During 2006 when conventional tube techniques were used for antibody screening and antibody identification 220 out of 18,627 (1.2%) patients were identified with positive antibody screens [Table 1]. Red cell alloantibodies were found in 0.9% (168/18,627 cases), antibodies of unclear specificity in 0.3% (51/18,627 cases) and autoantibodies in 16/18,627 (0.09%) cases [Table 1]. Twelve red cell alloantibody specificities were identified [Table 2]. In transfused patients, the five most frequent red cell antibody specificities identified by tube techniques were anti-Le a (31.4%), anti-Mi a (21.1%), anti-Le b (20.1%), anti-P a (11.3%) and anti-E (9.3%) respectively [Table 2]. Single alloantibodies were found in 135 (80.4%) patients, two in 31 (18.5%) and three or more were found in 2 (1.2%) alloimmunized patients (data not tabulated).

Similarly blood donor data using conventional tube techniques were reviewed for the same period. There were 20,786 blood donors tested and 0.9% (183/20,786) had a positive antibody screen [Table 1]. Antibodies were identified in 116/20,786 (0.6%) blood donors [Table 1]. Anti-Le a (57.2%), anti-Le b (29.0%), anti-Mi a (7.0%), anti-E (3.5%) and anti-P a (2.8%) were the five most common antibodies identified [Table 2]. 0.3% (67/20,786) of donors had an alloantibody of unclear specificity. Single alloantibodies were found in 87/116 donors (75%) and two antibodies in 29/116 donors (25%) (data not tabulated).

There were 27 patients (0.15%) who had immune alloantibody(ies) within the Rh, Duffy, Kidd, S and Diego systems and only 5 donors had an immune alloantibody in Rh system.

In both patient and donor populations, alloantibodies were found in 0.9% of subjects and overall the most frequent alloantibodies identified were anti-Le a (42.1%), anti-Le b (23.8%), anti-Mi a (15.2%), anti-P a (7.7%) and anti-E (6.9%) respectively [Tables 1 and 2].

For data from January 2008-December 2009 when CAT techniques were used for antibody screening, there was a total of 96,384 blood donors and patients tested. 566/96,384 (0.6%) donors and patients had a positive antibody screen and 422/566 had a specific antibody identified [Table 1].

Of the 47,155 patients tested, 468 (1%) had a positive antibody screen. Alloantibodies were identified in 0.8 % of patients

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**Table 1:** Frequency of antibodies detected in patients and donors by tube technique during Jan-Dec 2006 and CAT during Jan 2008-Dec 2009

| Tube technique Jan-Dec 2006 | Column agglutination technique (CAT) Jan 2008-Dec 2009 |
|-----------------------------|-------------------------------------------------------|
| Patients | Donors | Total | Patients | Donors | Total |
| N = 18,627 | N = 20,786 | N = 39,413 | N = 47,155 | N = 49,229 | N = 96,384 |
| Number of subjects with a positive antibody screen | 220 (1.2%) | 183 (0.9%) | 403 (1.0%) | 468 (1%) | 98 (0.2%) | 566 (0.6%) |
| • Number of subjects with alloantibody(ies) | 168 (0.9%) | 116 (0.6%) | 284 (0.7%) | 357 (0.8%) | 65 (0.13%) | 422 (0.44%) |
| • Number of subjects with alloantibodies of unclear specificity | 51 (0.3%) | 67 (0.3%) | 118 (0.3%) | 78 (0.17%) | 33 (0.07%) | 111 (0.12%) |
| • Number of subjects with autoantibodies | 16 (0.09%) | 0 | 16 (0.04%) | 103 (0.2%) | 0 | 103 (0.106%) |
| • Number of subjects with immune antibodies | 27 (0.15%) | 5 (0.02%) | 32 (0.08%) | 131 (0.28%) | 4 (0.008%) | 135 (0.14%) |
| Number of alloantibodies detected in each group | 204 | 145 | 349 | 484 | 74 | 558 |
| • Immune antibodies | 29 (14.2%) | 5 (3.4%) | 34 (9.7%) | 195 (40.3%) | 4 (5.4%) | 199 (35.7%) |
| • Natural antibodies | 175 (85.8%) | 140 (96.6%) | 315 (90.3%) | 289 (59.7%) | 70 (94.6%) | 359 (64.3%) |

Promwong, et al.: Red cell alloantibodies in the Southern Thais
(357/47,155), 0.2% of patients had autoantibody (103/47,155) and 0.17% of patients had an antibody of unclear specificity (78/47,155) [Table 1]. Sixteen red cell alloantibody specificities were identified. The five most frequent red cell alloantibodies were anti-Mi\(^b\) (32.9%), anti-E (17.1%), anti-Le\(^a\) (13.8%), anti-c (8.1%) and anti-Le\(^b\) (7.6%) respectively [Table 3]. Single alloantibodies were found in 76.8% patients, two alloantibodies were found in 17.9% and three or more in 5.3% of alloimmunized patients. Some patients had combinations of alloantibody(ies) and autoantibody or alloantibody(ies) and antibody of unclear specificity (data not tabulated).

There were 49,229 blood donors tested during Jan 2008-Dec 2009. 98 of the 49,229 donors (0.2%) had a positive antibody screen [Table 1]. Antibodies were identified in 65/49,229 (0.13%) donors and included anti-Le\(^a\) (39.2%), anti-Mi\(^b\) (29.7%), anti-Le\(^b\) (18.9%), anti-M (6.8%) and anti-D (4.1%). Antibodies of unclear specificity were found in 33/49,229 (0.07%). A single alloantibody was found in 56 donors and two alloantibodies in 9 donors (data not tabulated). In both patients and donors, 0.44% of subjects had alloantibodies and the most frequent alloantibodies detected were anti-Mi\(^b\) (32.4%), anti-Le\(^a\) (17.2%), anti-E (15.1%), anti-Le\(^b\) (9.1%), anti-c (7.0%) and anti-D (3.9%) respectively [Table 3].

There were 131 patients (0.28%) who had immune alloantibody(ies) within the Rh, Duffy, Kidd, S and Diego systems and only 4 donors had an immune alloantibody in Rh system.

**Discussion**

In the Southern Thai population when CAT was used for antibody detection and identification, the frequency of alloantibodies detected was 0.44%. Anti-Mi\(^b\), anti-Le\(^a\), anti-E, anti-Le\(^b\), anti-c and anti-D respectively were most frequently detected. While in the transfused patient population studied, the frequency of alloantibodies was 0.8% [0.28% were immune alloantibody(ies)]. In this group of subjects, the most frequent alloantibodies were anti-Mi\(^b\), anti-E, anti-Le\(^a\), anti-c, anti-Le\(^b\) and anti-D respectively.

When conventional tube techniques were used, the overall frequency of alloantibodies in patients and donors was 0.7% with the most frequent alloantibodies being anti-Le\(^a\), anti-Le\(^b\), anti-Mi\(^b\), anti-P, and anti-E respectively. 0.9% of transfused patients had alloantibodies and 0.15% of patients had immune alloantibody(ies). In this group, in order of frequency, the most common antibody specificities were anti-Le\(^a\), anti-Mi\(^b\), anti-Le\(^b\), anti-P, and anti-E respectively.

The alloantibody specificities detected by conventional tube technique were consistent with previous reports in Central Thai patients. In Central Thais, the frequency of detected alloantibodies was 4.91% and the most frequent alloantibodies were anti-Le\(^a\) (35.4%), anti-Le\(^b\) (28.36%), anti-P\(_1\) (21.83%), anti-Mi\(^b\) (6.4%) and anti-E (3.88%). Another study reported the frequency of alloantibodies ranging between 2.2 to 3.9%. Again anti-Le\(^a\) (26.3%), anti-Le\(^b\) (19.4%), anti-Mi\(^b\) (16.4%), anti-P\(_1\) (6.7%) and anti-E (5.3%) were the most common alloantibodies.

The frequencies of alloantibodies detected and the rank of antibody frequency in these studies were different from other studies. Possible explanations include different techniques, temperatures, and cells used for antibody screening and identification. Incubation at cooler temperatures would detect more cold reactive antibodies with fewer warm reactive alloantibodies. To our knowledge this would be the first report of alloantibody(ies) frequency in Thais using CAT for antibody screening and identification.

Following introduction of CAT for antibody screening and identification into our laboratory the incidence of anti-Mi\(^b\), anti-E, anti-c, Kidd and Duffy antibodies were more frequent than that associated with conventional tube techniques. Immune alloantibody(ies) were detected nearly twice as frequently when CAT was used compare to that of conventional tube techniques (0.28% v/s 0.15%). The reasons may include the increased

**Table 3: Red cell alloantibody specificities detected by CAT in patients and blood donors during Jan 2008-Dec 2009**

| Antibody Specificity | Patients with Antibodies (N = 360) | Donors with Antibodies (N = 65) | Total (N = 425) |
|----------------------|----------------------------------|-------------------------------|-----------------|
| **Antibody Frequency** | **1**                            | **1**                         | **2**           |
| E                   | 83 (17.1%)                       | 1 (1.4%)                     | 84 (15.1%)      |
| c                   | 3 (0.6%)                         | 3 (0.5%)                     | 6 (1.1%)        |
| D                   | 19 (3.9%)                        | 19 (3.9%)                    | 38 (7.1%)       |
| Le\(^a\)            | 67 (13.8%)                       | 67 (13.8%)                   | 134 (26.6%)     |
| Le\(^b\)            | 37 (7.6%)                        | 37 (7.6%)                    | 74 (15.5%)      |
| Jk\(^a\)            | 3 (0.6%)                         | 3 (0.6%)                     | 6 (1.2%)        |
| Jk\(^b\)            | 1 (0.2%)                         | 1 (0.2%)                     | 2 (0.4%)        |
| P\(_1\)             | 21 (11.3%)                       | 21 (11.3%)                   | 42 (8.9%)       |
| M                   | 3 (0.6%)                         | 3 (0.6%)                     | 6 (1.2%)        |
| N                   | 1 (0.2%)                         | 1 (0.2%)                     | 2 (0.4%)        |
| Mi\(^a\)            | 43 (21.1%)                       | 43 (21.1%)                   | 86 (17.3%)      |
| Di\(^a\)            | 2 (1.0%)                         | 2 (1.0%)                     | 4 (0.8%)        |
| **Total Antibodies**| 204                              | 145                           | 349             |

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sensitivity of CAT, or the improved quality of the antibody screen or panel cells. Anti-Le^a, anti-Le^b and anti-P^e were less common in CAT compared to tube techniques again this was likely due to incubation at 37°C only for CAT.

Anti-Mi^a is a very common antibody in Thais, Chinese and Taiwanese while there are no reports in Caucasians. The Miltenberger antigen (Mi^a) is commonly found in up to 15% of Chinese and South East Asian populations whereas it is far less common in other populations (<0.01%). Antibodies to variant MNS antigens (Mi^b) are common and behave like other MNS blood group antibodies. Most of these antibodies detected with Mi positive cells are IgM and tend to react best at cold temperatures. However, there are some that are IgG, active at 37°C reactive and clinically relevant. The frequency of anti-Mi^a in Central Thais was previously reported as 9.72%. In this study we found that anti-Mi^a was very common. The frequency of anti-Mi^a varied depending on the detection technique used. For tube techniques anti-Mi^a comprised 15.2% of alloantibodies detected, whereas when using CAT and screening cells that were Mi^a positive, anti-Mi^a was the most frequent antibody detected (32.4%). Again this difference may be due to different techniques and antigenic make-up of screening cells used. Anti-Mi^a is clinically significant with reports of haemolytic transfusion reaction (HTR) and haemolytic disease of the newborn (HDN). Therefore in Asian Mongoloid populations it is extremely important that Mi^a positive antibody screening cells and a number of those cells used for antibody identification are Mi^a positive. Anti-c and anti-E were also frequent immune alloantibodies detected in Southern Thais. Both of these antibodies are clinically significant and have been associated with haemolytic transfusion reactions and mild to moderate haemolytic disease of the newborn (HDN). Anti-D (3.9%) was not frequent in the Thai population due to the low frequency of RhD negative (<1.0%) within the Thais population. Although anti-D is rare in Thais, it often causes problems in clinical transfusion and severe haemolytic disease of the newborn because it is not well recognized by many clinicians.

There are differences in antibody specificities between Caucasian and Asian Mongoloid. Anti-K is a very clinically significant alloantibody in both clinical transfusion and haemolytic disease of the newborn and is found frequently in Caucasians but is very rare in Thai populations. In our study anti-K was not detected. Bejrachandra et al. found anti-K in only 1 out of 100,308 Central Thais tested. This may be due to the fact that most Thais are homogeneous kk and thus the rate of alloimmunization is very low.

Although there are increasing intermarriages between Thai women and Caucasian men there are no reports of HDN due to anti-K in the Thai population at this point in time. However, prenatal antibody screening is not included in the standard antenatal testing in most pregnancies throughout Thailand, the majority of pregnant women are only tested for ABO and RhD.

Anti-Jk^a and anti-Di^a were frequent in multiply transfused patients such as thalassemic patients in our study (unpublished observation). Di^a antigen is one of the antigens with low incidence among Caucasians but it is a relatively higher incidence among Asian-Mongoloid population. Anti-Di^a has been reported of causing haemolytic transfusion reaction and haemolytic disease of the newborn.

In this study antibodies were detected against antigens within the Rh, Kidd, Duffy, MNS and Diego systems. These are clinically significant alloantibodies (anti-D, -C, -c, -E, -e, -Fy^a, -Fy^b, -Jk^a, -Jk^b, -K, -M (active 37°C), S, -s, -U and -Di^a) and patients must receive antigen negative blood for transfusion and pregnant women screened for these antibodies due to the risk of HDN.

**Conclusion**

The overall frequency of alloantibodies in Southern Thais was 0.44% identified by CAT while the incidence in patients was 0.8%. The most frequent alloantibodies were anti-Mi^a, anti-Le^a, anti-E, anti-Le^b and anti-c. Anti-D and anti-Jk^a were also identified but less frequently while anti-K was not detected at all. Anti-Mi^a was very frequently identified in Southern Thais while anti-Di^a was not as common. However, Mi^a and Di^a antigens should be incorporated in antibody screening cell panels for Asian-Mongoloid population.

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