The Prevalence of Red Blood Cell Alloantibodies in Lower Northern Thailand

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Background: *Hemolytic transfusion reactions due to the presence of pre-existing alloantibodies are among the most common immunologic adverse effects in transfusion medicine. In addition to determining the presence and characteristics of the specific alloantibodies, one of the major obstacles is the selection of compatible blood units that lack the corresponding antigens to avoid such transfusion reactions. A delay in this process can lead to various detrimental complications.*
Objective: *To characterize the prevalence and specificity of alloantibodies in patients from lower northern Thailand who required a blood transfusion.*
Material and Method: *A retrospective review of the Blood Bank database of Naresuan University, Thailand, was conducted. Thirty one thousand four hundred patients who had been screened for the presence of alloantibodies between January 2007 and April 2014 were reviewed. The standard test tube method was used in all patients to identify the specificity of alloantibodies against red blood cell surface antigens.*
Results: *Among the 31,400 patients, 169 patients (0.54%) were found to have pre-existing red blood cell alloantibodies. Anti-Mi (anti-Miltenberger blood group) was the most common alloantibody identified (43.79%). Other common alloantibodies were anti-E (18.34%), anti-P_1 (17.75%), anti-Le^a (17.16%), and anti-Le^b (9.47%).*
Conclusion: *To minimize hemolytic transfusion reactions, we recommend greater availability and issuing of Mi, E, P_1, Le^a, and Le^b-matched blood units for patients with known alloantibodies against these antigens, or for patients who require multiple transfusions. This is in addition to the standard pre-transfusion screening and cross-matching processes.*

Keywords: *Red cell alloantibodies, Antibody specificity, Unexpected antibodies, Alloimmunization*

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Red blood cell alloantibodies are a common cause of transfusion complications[1] and problems in cross-matching of blood[2,3]. Overall, 35 blood group systems are recognized[4], however, only ABO and RhD antigens are routinely identified in pre-transfusion testing. A difference between red blood cell antigens of a donor and a transfused patient can result in the development of alloantibodies. If an unexpected antibody is found, more time is necessary to identify the antibody and to select the compatible corresponding antigen negative blood units. The prevalence of alloantibodies in different populations can predict the likelihood of finding compatible blood for a patient who has antibodies to red blood cells[1,2] and can be useful in preparing the most suitable negative blood group antigens that would be selected from inventory.

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In Thailand, data regarding the prevalence of alloantibodies are limited to patients in the central and southern areas of Thailand. There are no data available for patients in the lower northern area of Thailand. Naresuan University Hospital is a tertiary care hospital located in lower northern Thailand, which supports patients in nine surrounding provinces. This background study was undertaken to characterize the prevalence and specificity of alloantibodies in patients from the lower northern area of Thailand who required a blood transfusion.

Material and Method

A retrospective review of the Blood Bank database of Naresuan University Hospital was conducted in September, 2014. Thirty one thousand four hundred patients who had been screened for the unexpected antibodies between January 2007 and April 2014 were included in the present review. Patients with missing data were excluded from the study. We sought blood groups and alloantibodies obtained from antibody screening and identification, and, the

essential data such as gender, domicile, and medical history.

Antibody screening and identification was performed by the standard test tube methods in all patients who had the unexpected antibodies. A set of 2-screening O cell suspensions, a set of 11-antibody identification panel cells (3% cells suspended in preservative solution which was manufactured by the Thai National Blood Centre) and polyspecific anti-human globulin reagent (containing anti-IgM, anti-IgG, and anti-c3d) were provided by the National Blood Centre of Thai Red Cross. Blood group antigens in all red cell reagents were indicated including D, C, c, E, e, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, Mi^a, M, N, K, k, S, s, P_1, Lu^a, Lu^b, and Di^a. The indirect antiglobulin technique was used for antibody screening and antibody identification which was incubated at room temperature phase, 37°C phase and indirect antiglobulin phase. If patient had a positive antibody screening result in any phase, his or her serum was further tested with his or her own red cells (called autocontrol) and it was identified the specificity of antibody. If autocontrol was negative, patient's antibody was identified as alloantibody. Autoantibody was indicated in patient who had positive autocontrol and had been further confirmed by positive direct antiglobulin test. The data were analyzed by using descriptive statistics. The protocol of the present study was approved by the Naresuan University Institutional Review Board, IRB No. 347/57.

Results

Among the 31,400 patients included in the present review, we found 176 patients (0.56%) with positive results of the antibody screening test. In the 176 positive antibody screening patients, 72 (40.91%) were male, 104 (59.09%) were female. The distributions of ABO and Rh(D) blood groups revealed 61 (34.66%) were O, 43 (24.43%) were A, 65 (36.93%) were B, 7 (3.98%) were AB, 175 (99.43%) were Rh(D) positive, and only one (0.57%) was Rh(D) negative. Patients with positive antibody screening were further tested for antibody identification and autocontrol. Of the 176 patients, 169 patients had alloantibodies against red blood cells, whereas seven patients had autoantibodies alone. The prevalence of alloantibodies was 0.54%.

With regard to the medical histories, alloimmunized patients were grouped into five departments, which were Medicine, Pediatrics, Surgery, Orthopedics, and Obstetrics and Gynecology (shown in Table 1). Of interest, it seemed that patients receiving care at the department of Medicine were the majority group of alloimmunized patients. Of these 107 medical patients had 139 alloantibodies. The disease in this

Table 1. The finding of alloimmunized patients categorized by departments and medical conditions

Departments	Number of alloimmunized patients	Most frequent medical conditions
Medicine	107 (63.32%)	Thalassemia (14.95%), CKD (10.28%), UGIH (9.35%), HCC (6.54%), MDS (3.74%), others* (55.14%)
Surgery	25 (14.79%)	Vulvular heart disease (72.00%), neurosurgery related (12.00%), BPH (4.00%), intestinal obstruction (4.00%), injury of spleen (4.00%), chronic venous insufficiency (4.00%)
Orthopedics	23 (13.61%)	Arthrosis of the knee (39.13%), fracture (39.13%), spine surgery related (13.04%), osteonecrosis (8.70%)
OB/GYN	12 (7.10%)	Antenatal screening (50.00%), myoma uteri (16.67%), endometriosis (16.67%), abortion (8.33%), adenomyosis (8.33%)
Pediatrics	2 (1.18%)	Anemia (50.00%), UGIH (50.00%)

OB/GYN = obstetrics and gynecology; CKD = chronic kidney disease; UGIH = upper gastrointestinal hemorrhage; HCC = hepatocellular carcinoma; MDS = myelodysplastic syndrome

* Malignant neoplasm of ovary 3.74%, malignant neoplasm of colon 3.74%, immune thrombocytopenic purpura 2.80%, cirrhosis of liver 2.80%, anemia 1.87%, aortic valve stenosis 1.87%, atrial fibrillation 1.87%, mitral stenosis 1.87%, aplastic anemia 1.87%, autoimmune hemolytic anemia 1.87%, malignant neoplasm of lung and bronchus 1.87%, lymphoma 1.87%, multiple myeloma 1.87%, renal insufficiency 0.93%, heart failure 0.93%, iron deficiency anemia 0.93%, ischemic cardiomyopathy 0.93%, intestinal obstruction 0.93%, intracerebral hemorrhage 0.93%, pulmonary tuberculosis 0.93%, anemia of chronic disease 0.93%, gastrointestinal stromal tumor 0.93%, systemic lupus erythematosus 0.93%, infective endocarditis 0.93%, chronic viral hepatitis 0.93%, nephritic syndrome 0.93%, stroke 0.93%, hypertension 0.93%, thrombotic microangiopathy 0.93%, cerebral infarction 0.93%, atherosclerotic heart disease 0.93%, ulcerative colitis 0.93%, malignant neoplasm of endometrium 0.93%, acute myeloid leukemia 0.93%, malignant neoplasm of breast 0.93%, cervical carcinoma 0.93%, malignant neoplasm of thymus 0.93%, malignant neoplasm of bladder 0.93%, malignant neoplasm of liver 0.93%, malignant neoplasm of hypopharynx 0.93%

group were thalassemia (14.95%), followed by chronic renal failure (10.28%), upper gastrointestinal hemorrhage (9.35%), liver cell carcinoma (6.54%), myelodysplastic syndrome (3.74%), and others (55.14%).

Overall 208 alloantibodies were detected in the 169 patients. Of the 208 alloantibodies, 199 were identified by their specific characteristics whereas 9 were unidentified. One hundred forty six patients (86.39%) had single alloantibodies, 13 patients (7.69%) had two alloantibodies whereas the other 10 patients (5.92%) had more than two alloantibodies (shown in Table 2). The most frequent occurring specificity of red blood cell alloantibodies in patients with single alloantibody was anti-Mi. In multiple alloimmunized patients, anti-E with anti-Mi were the most frequent, followed by anti-E + anti-c and anti-Le^a + anti-Le^b. Only one patient, who had thalassemia, had all 5 alloantibodies; anti-E, anti-c, anti-Mi, anti-Jk^a, and anti-S. The medical history of the multiple alloimmunized patients were reviewed and 17 had a medical condition, for example thalassemia or liver cell carcinoma. Only 6 had surgical conditions such as bone fracture, rupture of the spleen, and valvular heart disease.

All 12 types of alloantibodies were indicated, with anti-Mi the most frequent (37.19%), anti-E (15.58%), anti-P_1 (15.08%), anti-Le^a (14.57%), anti-Le^b (8.04%), anti-c (3.02%), anti-Jk^a (2.01%), anti-Jk^b (1.51%), anti-N (1.01%), anti-S (1.01%), anti-D (0.50%), and anti-Di^a (0.50%) (shown in Table 3).

Discussion

In the present study, the prevalence of alloantibodies was 0.54%. These data were lower than in previous studies in different regions of Thailand. Possible explanations of different prevalence rates in each regions were different characteristics of patients, varied antibody screening protocols and different panel cells for antibody identification[1,2]. In 1979, the study of patients in Central Thailand (Bangkok area and surrounding provinces) revealed the frequency of alloantibodies was 4.91%[5]. In another study in Central Thailand, the frequency of alloantibodies was 2.2 to 3.9%[6]. Recently, a study of patients in Southern Thailand revealed that by using standard test tube methods, the frequency of alloantibodies was 0.9% and autoantibodies was 0.09%[7].

In the present study, most patients had a single alloantibody rather than multiple alloantibodies (Table 2). The highest frequency alloantibody was anti-Mi. This differs from other Asian studies[2,5,7,8] but are similar with a Malaysian study[1]. However, anti-E was the second most common antibody found in the present study and the highest frequency was reported, in most studies, in populations of Han Chinese, Indians, and Kelantan Malaysians[2,3,8]. Table 3 showed the ranking of alloantibody frequency from the present study, compared to other studies. All five antibodies such as anti-Mi, anti-E, anti-P_1, and anti-Lewis were the most frequent in patients in the Lower Northern Thailand area, which is similar to the results from other Thai studies[5-7]. We noticed that all these five alloantibodies are the most common alloantibodies among Thai patients but this is quite different from what has been found in Malaysian, Chinese, and Indian patients[1,2,8]. Comprehending this statement is essential for preventing transfusion reactions when phenotyping all these 5 corresponding antigens in Thai patients.

Table 2. Specificity of red blood cell alloantibodies in lower northern Thai patients

Single alloantibodies	Number	Two alloantibodies	Number	More than 2 alloantibodies	Number
Anti-Mi	65	Anti-E + anti-Mi	4	Anti-E + anti-c+ anti-Mi	1
Anti-P_1	28	Anti-Le^a + anti Le^b	3	Anti-E + anti-P_1 +unknown	1
Anti-Le^a	22	Anti-Le^a + unknown	2	Anti-E + anti-Jk^a + unknown	1
Anti-E	16	Anti-Mi + anti-Le^b	1	Anti-Le^a + anti-Le^b + anti-P_1	1
Anti-Le^b	10	Anti-E + anti Jk^a	1	Anti-Le^a + anti-Le^b + unknown	1
Anti-N	2	Anti-E + anti-S	1	Anti-E + anti-c + anti-Mi + anti-Jk^b	1
Anti-D	1	Anti-E + anti-c	1	Anti-E + anti-c + anti-Mi + unknown	1
Anti-Jk^a	1			Anti-E + anti-c + anti-Jk^b + unknown	1
Unknown	1			Anti-E + anti Jk^a + anti-Di^a + unknown	1
				Anti-E + anti-c + anti-Mi + anti-Jk^b + anti-S	1
Total (% of total 31,400 patients)	146 (0.47%)		13 (0.04%)		10 (0.03%)

Table 3. Distribution of specificity of alloantibodies among lower north of Thai patients compared with other studies

	Lower North of Thailand (n = 169)	Southern of Thailand Promwong et al.[7] (n = 167)	Malaysia Yousuf et al.[1] (n = 142)	China Xu et al.[8] (n = 212)	India Makroo et al.[2] (n = 239)
Anti-Mi	74 (37.19%)	43 (21.10%)	49 (32.45%)*		
Anti-E	31 (15.58%)	19 (9.30%)	21 (13.91%)	100 (42.37%)	89 (29.27%)
Anti-P_1	30 (15.08%)	23 (11.30%)	8 (5.30%)		
Anti-Le^a	29 (14.57%)	64 (31.40%)	5 (3.31%)	6 (2.54%)	3 (0.98%)
Anti-Le^b	16 (8.04%)	41 (20.10%)	2 (1.32%)		2 (0.65%)
Anti-c	6 (3.02%)	2 (1.00%)	9 (5.96%)	13 (5.51%)	24 (7.89%)
Anti-Jk^a	4 (2.01%)	3 (1.50%)	10 (6.62%)		13 (4.27%)
Anti-Jk^b	3 (1.51%)	1 (0.50%)	1 (0.66%)	5 (2.12%)	10 (3.28%)
Anti-N	2 (1.01%)	1 (0.50%)	3 (1.99%)	5 (2.12%)	9 (2.96%)
Anti-S	2 (1.01%)		4 (2.65%)	2 (0.85%)	7 (2.30%)
Anti-D	1 (0.50%)	2 (1.00%)	22 (14.57%)	45 (19.07%)	46 (15.13%)
Anti-Di^a	1 (0.50%)	2 (1.00%)	1 (0.66%)		
Anti-M		3 (1.50%)	3 (1.99%)	44 (18.64%)	24 (7.89%)
Anti-MUT			7 (4.64%)		
Anti-e			2 (1.32%)	6 (2.54%)	2 (0.65%)
Anti-C			2 (1.32%)	5 (2.12%)	26 (8.55%)
Anti-Fy^b			1 (0.66%)		2 (0.65%)
Anti-Mur			1 (0.66%)	3 (1.27%)	
Anti-Fy^a				2 (0.85%)	8 (2.63%)
Anti-K					30 (9.86%)
Anti-C^w					7 (2.30%)
Anti-V					1 (0.32%)
Anti-Kp^a					1 (0.32%)
Total antibodies	199	204	151	236	304

* Anti-Mi^a

Only 1 patient was observed with anti-D in the present study, whereas other authors have reported that anti-D was the second most commonly observed antibody especially in Asia[1,2,8]. The possible reason is the very low prevalence of the RhD negative patients in Thailand. We found anti-c was the sixth most common in the present study. Anti-c was not observed in the single-alloantibody group, although it was in the multiple-alloantibody group, usually accompanied by anti-E. Other than anti-E, anti-Mi was one of the potentially clinically significant antibodies and it was the highest frequent in the present study. Sixty percent of detected anti-Mi were a warm-active IgM and monocyte-monolayer-assay positive-IgG according to the previous study[9]. Many studies have reported that anti-Mi is associated with hydrops fetalis, hemolytic transfusion reactions[9-12].

In the present study, anti-Mi was used for replacement of an obsolete name 'anti-Mi^a' which has been used in previous Thai studies. Anti-Mi^a remains in colloquial use in Thailand, which refers to an antibody that reacts with a group of red cell antigens of the Miltenberger (Mi) series. So far, up to now, 11 subclasses (Mi.I to Mi.XI) have been defined in the Miltenberger series[11], but determining the specificity of anti-Mi can be performed further by known Mi-subclasses of red blood cell panels. Subclasses of anti-Mi were not identified in the present study because of limited availability of those red blood cell panels. However, most of the screening cells provided by the National Blood Centre of the Thai Red Cross were Mi.III subclasses which were common in Thai people (sometimes Mi.II or Mi.VI were added)[13]. In addition, we could imply that anti-Mi was an antibody to groups

of antigens in Mi.III (GP Mur), Mi.II (GP Hut), and Mi.VI (GP Bun).

Although anti-P_1 and anti-Lewis are usually cold-reactive antibodies without clinical significance, some authors have reported cases of hemolytic transfusion reaction that were attributed to these antibodies[14-17]. One study mentioned that half of the anti-Lewis antibodies were IgG isotype, so they had potential clinical significance[18]. In the present study showed anti-P_1 and anti-Lewis were common antibodies. Furthermore, anti-Lewis and anti-P_1 were prevalent in other area of Thailand to a high degree[5-7] which can lead to difficulty in cross-matching and can produce various adverse reactions. Thus, if anti-Lewis or anti-P_1, which are known to be thermally active at 37°C, were detected, the corresponding antigen negative blood units must be provided.

The distribution of departments showed that non-surgical patients had developed alloantibodies and autoantibodies at a higher level than surgical patients. We found that the higher proportions of alloantibodies were in patients with a medical disease such hematologic disorders and cancers. This too was similar finding to other report[3]. This latter finding provides an explanation as to why repetitive transfusions needed in chronic medical conditions are more prone to exposing allogeneic antigens than single transfusions usual in surgical conditions.

Although previous studies suggest that issuing an antigen negative or phenotype matched blood unit is the most appropriate for preventing the alloimmunization in transfused patients[1,3,7], in Thailand, this recommendation has not been applied in practice. Due to the limitation of budget, red cell phenotyping is usually performed only in frequent donors or frequent transfused thalassemic patients. The solution that we propose is to understand the prevalence of alloantibodies in our community and select only top 5 - the Mi, E, P_1, Le^a, and Le^b - negative matched blood donors, which would significantly decrease the duration of blood component cross matching and prevent adverse clinical consequences.

Conclusion

Anti-Mi, followed by anti-E, anti-P_1, anti-Le^a, and anti-Le^b were the top five alloantibodies in the present study. Hence, we recommend the known prevalent red blood cell antigens in local donors to prepare all these five antigen negative blood units for inventory and issuing Mi, E, P_1, Le^a, and Le^b - matched blood units for patients with known alloantibodies against these antigens, or patients who require multiple transfusions. Furthermore, the need to confirm the presence of anti-Mi subclasses, as well as to phenotype the Miltenberger (Mi) antigens, should be a concern in Thailand.

What is already known on this topic?

In repetitive transfused patients, as chronic medical conditions, are more prone to expose to allogeneic antigens than single transfusion usual in surgical conditions. Patients with multiple alloantibodies were frequently found in patients receiving care at the department of Medicine.

What this study adds?

This study presented the first report of prevalence and characteristic of alloantibodies in patients from lower northern Thailand. Preparing Mi, E, P_1, Le^a, and Le^b - antigen negative blood units for inventory and issuing all five antigens - matched blood units for patients who required multiple transfusions is the additional step to minimize hemolytic transfusion reactions.

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Potential conflicts of interest

None.

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ความชุกของการสร้างแอนติบอดีต่อหมู่เลือดในผู้ป่วยแถบภาคเหนือตอนล่างของประเทศไทย

พสุพร โพธิ์เงินนาค, สุธิดา ศาสตร์จีนพงษ์, กาญจนาพร เชื้อสกุล, กุลนิษฐ์ แพงวังทอง

ภูมิหลัง: *ภาวะเม็ดเลือดแดงแตกจากการมีแอนติบอดีต่อเม็ดเลือดแดงนั้นเป็นผลข้างเคียงที่พบบ่อยในเวชศาสตร์ธนาคารเลือด นอกจากความจำเป็นที่ต้องตรวจหาแอนติบอดีในผู้ป่วยนั้นให้พบแล้ว ปัญหาสำคัญอย่างหนึ่งคือ การจัดหาเลือดที่มีหมู่เลือดไม่ตรงกับแอนติบอดีในเลือดของผู้ป่วยเพื่อป้องกันความเสี่ยงที่จะเกิดภาวะเม็ดเลือดแดงแตกหลังจากการรับเลือดเข้าไป ส่งผลให้ขั้นตอนการจัดหาเลือดให้แก่ผู้ป่วยกลุ่มนี้จะล่าช้ากว่าปกติ*

วัตถุประสงค์: *เพื่อศึกษาความชุกและชนิดของแอนติบอดีต่อหมู่เลือดในผู้ป่วยแถบภาคเหนือตอนล่างของประเทศไทย*

วัสดุและวิธีการ: *การศึกษาแบบสำรวจข้อมูลย้อนหลังของผู้ป่วยที่เข้ารับการตรวจคัดกรองและระบุชนิดแอนติบอดีต่อหมู่เลือดในงานธนาคารเลือด โรงพยาบาลมหาวิทยาลัยนเรศวร ระหว่างเดือนมกราคม พ.ศ. 2550 ถึง เมษายน พ.ศ. 2557 ซึ่งช่วงเวลาดังกล่าวการตรวจคัดกรองและหาชนิดของแอนติบอดีต่อเม็ดเลือดแดงใช้วิธีหลอดทดลองมาตรฐาน*

ผลการศึกษา: *ในช่วงเวลาดังกล่าวมีผู้ป่วยที่เข้ารับการตรวจคัดกรองแอนติบอดีทั้งสิ้น 31,400 ราย พบว่ามีการสร้างแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยจำนวน 169 ราย (ร้อยละ 0.54) เมื่อจำแนกชนิดของแอนติบอดี พบว่า anti-Mi (anti-Miltenberger blood group) พบบ่อยที่สุด (ร้อยละ 43.79) รองมาคือ anti-E (ร้อยละ 18.34), anti-P_1, (ร้อยละ 17.75), anti-Le^a (ร้อยละ 17.16) และ anti-Le^b (ร้อยละ9.47)*

สรุป: *เพื่อลดความเสี่ยงต่อการเกิดปฏิกิริยาหลังการรับเลือดในผู้ป่วย จึงแนะนำให้สำรองเลือดที่ไม่มีแอนติเจนในหมู่ Mi, E, P_1, Le^a และ Le^b เพิ่มขึ้นให้เพียงพอสำหรับผู้ป่วยที่ต้องการใช้เลือดที่ทราบแล้วว่ามีแอนติบอดีต่อหมู่เลือดเหล่านี้อยู่ หรือ ผู้ป่วยที่มีความจำเป็นต้องใช้เลือดบ่อยครั้ง นอกเหนือจากการตรวจหาหมู่เลือดก่อนการให้เลือดและตรวจความเข้ากันได้ของเลือดด้วยวิธีมาตรฐานที่ได้ทำกันอยู่ทั่วไป*