Original Article

Analysis of pretransfusion testing events occurring in patients at Thammasat University Hospital

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Abstract:

Introduction: *ABO grouping and Rh(D) typing, antibody screening, and crossmatching are essential components of pretransfusion testing before blood transfusion in patients. Various problems that occurred in pretransfusion testing caused delayed resolutions before blood can be released for patients. This study aimed to analyze the pretransfusion testing problems in patients who requested transfusions at Thammasat University Hospital* ***Meteria ls and Methods:*** *This retrospective study was conducted at the Blood Bank, Thammasat University Hospita1 from January 2019 to December 2019. Data regarding sex, age, transfusion, pregnancy history and pretransfusion testing results were collected and analyzed.* ***Results:*** *Totally, 23,659 patients' transfusion requests were evaluated. One case of ABO discrepancy was due to the extra cold alloantibodies of the Lewis system. The prevalence of unexpected red cell antibodies was 2.46% (581/23,659) and group B patients were significantly higher than other blood groups. More than 90% of cases could be identified antibody specificity in our laboratory and the most common alloantibodies were anti-Mi^{a}, anti-E, and anti-c. The other undetermined specificities were due to autoantibody combined with a mixture of alloantibodies and unidentified antibodies, which could provide either compatible or least incompatible phenotype-matched blood without any signals of transfusion reactions.* ***Conclusion:*** *These findings in patients represented potentially clinically relevant data on red cell antibodies. The association with frequently observed alloantibodies is predictive of the appropriate management of antigen-negative blood to ensure prompt and safe blood transfusions.*

Keywords : ● *Pretransfusion testing* ● *Patients* ● *Alloantibodies*

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นิพนธ์ต้นฉบับ

การวิเคราะห์ปัญหาของการตรวจก่อนการให้เลือดที่พบในผู้ป่วย ณ โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ

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บทคัดย่อ

บทนำ *การตรวจหมู่เลือด ABO การตรวจ Rh(D) การตรวจกรองแอนติบอดีและการตรวจความเข้ากันได้ของเลือดเป็นส่วนประกอบสำคัญของการตรวจก่อนการให้เลือดกับผู้ป่วย ปัญหาต่าง ๆ ที่เกิดขึ้นในการตรวจก่อนการให้เลือดทำให้เกิดความล่าช้าทั้งการแก้ปัญหาและการจ่ายเลือดให้กับผู้ป่วย* ***วัตถุประสงค์*** *การศึกษานี้เพื่อวิเคราะห์ปัญหาต่าง ๆ ที่เกิดขึ้นในการตรวจก่อนการให้เลือดในผู้ป่วยที่ขอเลือด ณ โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ* ***วัสดุและวิธีการ*** *เป็นการศึกษาย้อนหลังที่ธนาคารเลือด โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ ตั้งแต่เดือนมกราคม พ.ศ. 2562 ถึงเดือนธันวาคม พ.ศ. 2562 โดยรวบรวมและวิเคราะห์ข้อมูลของเพศ อายุ ประวัติการได้รับเลือดและการตั้งครรภ์ รวมทั้งผลการตรวจก่อนการให้เลือด* ***ผลการศึกษา*** *ได้ประเมินข้อมูลผู้ป่วยที่ขอเลือดจำนวนทั้งหมด 23,659 ราย พบว่า ผู้ป่วย 1 ราย มีปัญหา ABO discrepancy เนื่องจากมีแอนติบอดีของหมู่เลือดระบบ Lewis ความชุกของการตรวจพบแอนติบอดีเท่ากับ 2.46% (581/23,659) และผู้ป่วยหมู่ B ตรวจพบแอนติบอดีสูงกว่าผู้ป่วยหมู่อื่นอย่างมีนัยสำคัญ ห้องปฏิบัติการสามารถตรวจแยกชนิดของแอนติบอดีได้มากกว่า 90% แอนติบอดีที่พบได้บ่อยคือ anti-Mi^{a}, anti-E, และ anti-c ส่วนแอนติบอดีที่ไม่สามารถแยกชนิดได้ ได้ส่งไปตรวจที่ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย พบว่า เป็น autoantibody ร่วมกับ alloantibodies อื่นและ unidentified antibodies ซึ่งสามารถให้เลือดที่เป็น phenotype-matched ทั้งที่เป็น compatible และ least incompatible โดยที่ไม่พบอาการแสดงของปฏิกิริยาอันไม่พึงประสงค์จากการได้รับเลือด* ***สรุป*** *จากผลการศึกษาในผู้ป่วยพบว่า ปัญหาส่วนใหญ่เกิดจากแอนติบอดีต่อหมู่เลือดที่มีความสำคัญทางคลินิก ดังนั้นความสัมพันธ์ของการตรวจพบ alloantibodies ชนิดที่พบได้บ่อยเป็นตัวบ่งชี้ในการบริหารจัดการคัดเลือกเลือดที่เป็น antigen-negative ให้เหมาะสมเพื่อยืนยันการให้เลือดที่ปลอดภัยและทันเวลากับผู้ป่วย*

คำสำคัญ : ● *การตรวจก่อนการให้เลือด* ● *ผู้ป่วย* ● *Alloantibodies*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2563;30:345-51.

Introduction

To prevent incompatible blood transfusions, pretransfusion testing including ABO grouping and Rh(D) typing, antibody screening test and crossmatching are essential for transfusion candidates.[1] Regarding the National Blood Centre, Thai Red Cross Society (NBC-TRCS) guidelines, a patient with nebative antibody screening, ABO and Rh compatible blood can be selected for crossmatching. The crossmatching procedures must primarily detect ABO incompatibility and antibodies against donor red cells including immediate-spin, 37°C and indirect antiglobulin test (IAT).[2] In cases of positive antibody screening, antibody identification should be performed not only to determine antibody specificity but also to provide donor blood that lacks the corresponding antigen to the patients.[1-3]

Problems in ABO discrepancies are usually encountered in phlebotomy errors, patient misidentification and immunohematologic findings.[4] A patient's history must be checked to compare current and previous results. The information must be obtained to explain the reason of the discrepancies such as stem cell transplantation and previous transfusions. Moreover, discrepancies may arise from intrinsic problems with the red cells, plasma, or from technical errors in performing the test.[5] Hence, ABO testing discrepancies must be resolved before selecting group-specific donor units.

Although the prevalence of Rh negative in Thai populations has been estimated to be 0.1-0.3%.[6,7] The D antigen is the strongest immunogenicity resulting in a subsequent anti-D formation after transfusion or pregnancy. A related study in multitransfused Thai patients, anti-D and anti-D combined with other alloantibodies have been found 26 and 16 (1.47% and 0.91%) in 1,766 patients' samples, respectively.[8] Importantly, transfusing Rh-negative donor units to either Rh-negative or weak D patients is recommended to reduce the risk of alloimmunization.[9]

Antibody screening test in patients receiving blood transfusions is beneficial to detect alloantibodies which are the leading cause of hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN). The frequencies of red cell alloantibodies vary in populations, 0.8% in blood donors; 2.9% in patients with a history of blood transfusions, and 9 to 30% among patients receiving repeated blood transfusions.[10,11] When the antibody screening test is positive, antibody identification is performed to identify antibody specificity(ies). Then, antigen-negative blood must be selected for crossmatching. In a patient who has a mixture of autoantibody and alloantibodies, the direct antiglobulin test (DAT) and the autocontrol will show positive results. This problem causes delayed resolutions and blood transfusions. If transfusion is necessary before compatible blood can be obtained, the decision of transfusing potentially incompatible crossmatch is based on the clinical condition of the patients.[10] This retrospective study aimed to analyze the pretransfusion testing problems in patients at Thammasat University Hospital.

Materials and Methods

This retrospective study was conducted at the Blood Bank, Thammasat University Hospital from January 2019 to December 2019. Data regarding sex, age transfusion and pregnancy history were collected. ABO grouping, and Rh(D) typing, antibody screening, and crossmatching were performed by column agglutination technology (CAT) using an ORTHO VISION Analyzer (Ortho-Clinical Diagnostics, Raritan, NJ, USA). Weak D confirmation test was performed by indirect antiglobulin test, IAT (Bio-Rad, Cressier, Switzerland). In case of the patient's plasma showed positive antibody screening, antibody identification was performed using 11-panel cells (NBC-TRCS, Bangkok, Thailand) along with an auto control. A DAT was performed in all cases with positive autocontrol. All tests were performed according to the standard operating procedures and the manufacturer instructions.

Statistical analysis

Descriptive analysis of antibody screening results obtained from all patients was performed according to sex and ABO blood groups. The results were expressed in percentages and 95% confidence intervals (CI), and odds ratios of ABO blood groups from these positive and negative antibody screening results were compared. The results were analyzed using SPSS 16.0 Software (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant.

Results

During the period of study, a total of 23,659 patients at the Blood Bank, Thammasat University Hospital were evaluated. All patients comprised of 10,354 males and 13,305 females (age range from 1 year to 99 years). For ABO types, group O was the most common (37.55%), followed by group B (35.09%), group A (20.62%), and group AB (6.74%), respectively, as shown in Table 1. For Rh(D) types, Rh positive patients were 23,608 (99.79%), Rh negative patients were 46 (0.19%) and 5 remaining patients were weak D (0.02%).

ABO discrepancy was observed in a male patient. The cell grouping showed B and serum grouping found as similar to O with the reactions (4+) with screening O1 and O2 cells. The patient's red cell agglutinated with monoclonal anti-H (NBC-TRCS, Bangkok, Thailand), hence, the para-Bombay phenotype was ruled out. Additionally, antibody screening cells reacted at room temperature and indirect antiglobulin test (IAT) showed the presence of anti-Le^a + -Le^b in the patient's plasma. Phenotype of the patient's red cells showed the absence of Le^a and Le^b antigens. The conclusion was made that this patient was group B.

Among 23,659 patients, the prevalence of irregular red cell antibodies was 2.46% (581/23,659); 95%CI: 0.0227-0.0267. The frequencies of positive antibody screening test results were higher in females (1.58%) than male patients (0.88%) and no significant difference was found in different age groups. The distribution of ABO blood group and frequencies of positive antibody screening test results were evaluated. The patients with group B were significantly higher than those of other blood groups (OR = 1.3401, 95%CI: 1.1339-1.5837, p = 0.0006), as shown in Table 1.

Regarding the antibody specificities found among 581 patients with positive antibody screening test results (Table 2), 426 (73.32%) patients had a single antibody, 46 (7.92%) had two antibodies, 44 (7.57%) had three antibodies and 9 (1.55%) had more than three antibodies.

Table 1 Distribution of antibody screening test results of 23,659 patients with transfusion requests according to sex and ABO groups

ABO group	Number of patients (%)	Number of antibody screening test results (%)					
		Positive			Negative		
		Total	Male	Female	Total	Male	Female
A	4,878	96	41	55	4,782	2,066	2,716
	(20.62)	(0.41)	(0.17)	(0.23)	(20.21)	(8.73)	(11.48)
B	8,301	243*	86	157	8,058	3,652	4,406
	(35.09)	(1.03)	(0.36)	(0.67)	(34.06)	(15.44)	(18.62)
O	8,885	206	72	134	8,679	3,727	4,952
	(37.55)	(0.87)	(0.30)	(0.57)	(36.68)	(15.75)	(20.93)
AB	1,595	36	10	26	1,559	700	859
	(6.74)	(0.15)	(0.04)	(0.11)	(6.59)	(2.96)	(3.63)
Total	23,659	581	209	372	23,078	10,145	12,933
	(100.00)	(2.46)	(0.88)	(1.58)	(97.54)	(42.88)	(54.66)

*OR = 1.3401, 95%CI: 1.1339-1.5837, p = 0.0006

Table 2 Red cell antibody frequencies and specificities encountered among 581 patients

Antibody specificity	Number	%
Single antibody	**426**	**73.32**
Anti-Mi^a	299	51.46
Anti-E	74	12.74
Anti-Le^a	27	4.65
Anti-Di^a	10	1.72
Anti-c	5	0.86
Anti-Jk^a	3	0.52
Anti-M	3	0.52
Anti-P1	2	0.34
Anti-D	1	0.17
Anti-Jk^b	1	0.17
Anti-K	1	0.17
Two antibodies	**46**	**7.92**
Anti-E + -c	19	3.28
Anti-E + -Mi^a	15	2.58
Anti-E + -Di^a	4	0.69
Anti-Mi^a + -Di^a	3	0.52
Anti-Mi^a + -Jk^a	2	0.34
Anti-M + -Mi^a	2	0.34
Anti-c + -Mi^a	1	0.17
Three antibodies	**44**	**7.57**
Anti-E + -c + -Mi^a	32	5.51
Anti-E + -Mi^a + -Di^a	6	1.03
Anti-E + -N + -P1	4	0.69
Anti-C + -e + - Jk^a	1	0.17
Anti- E + -Mi^a + -Jk^b	1	0.17
More than three antibodies	**9**	**1.55**
Anti-E + -c + -Mi^a + -Le^b	3	0.53
Anti-E + -c + -Mi^a + -M	2	0.34
Anti-E + -c + -Mi^a + -S	2	0.34
Anti-E + -c + -Mi^a + -Le^a + -Jk^a	2	0.34
Inconclusive results*	**56**	**9.64**
Total	581	100.00

*The samples were sent to NBC-TRCS

In addition, inconclusive results were demonstrated among 56 (9.64%) patients and those patients' samples were sent to NBC-TRCS for further investigation. For the patients with a single antibody, anti-Mi^a was the most common (51.46%), followed by anti-E (12.74%), anti-Le^a (4.65%) and anti-Di^a (1.72%), respectively. The anti-E + -c and anti-E + -Mi^a were commonly found in multiple antibodies.

The results of antibody identification in 56 patients with history of multiple transfusions (~5.77 times) were obtained from NBC-TRCS (Table 3). Fifty-three patients showed positive autocontrol results, and most of them were combined with a mixture of alloantibodies either identified or unidentified antibody specificities. The three remaining patients showed negative autocontrol results, all were a mixture of anti-E + -c and other alloantibodies. Among 56 patients, compatible crossmatch antigen-negative donor red cell units were transfused among 33 patients (58.92%). Least incompatible crossmatch antigen-negative blood could be observed in remaining 23 patients; however, only 13 patients (23.21%) were transfused and 10 (17.86%) did not receive transfusion.

Discussion

This study was undertaken to analyze the pretransfusion testing problems among patients who requested transfusion at Thammasat University Hospital to supply appropriate blood for the typical problem-patient groups. The distribution of ABO blood groups among 23,659 patients was analyzed, and the results were similar to a related study conducted among Thai blood donors.[7,12,13] Thus, the management of our stock inventory could be maintain between the demand and supply ratio of the four blood groups. In the case of ABO discrepancy, the extra plasma reactivity was demonstrated and confirmed as anti-Le^a and -Le^b. The patient phenotype was Le(a-b-) and 4 units of group B, Le(a-b-) compatible crossmatch were transfused without transfusion reactions. Finding the Le(a-b-) in Thai blood donors is possible because this phenotype is about 20-30%.[6,12,13]

Table 3 Red cell antibody frequencies and specificities results obtained from NBC-TRCS among 56 patients

Antibody specificity	Number	%
Mixture of alloantibodies		
Anti-E + -c + Jk^b	1	1.79
Anti-E + -c + Le^b	1	1.79
Anti-E + -c + Mi^a + unidentified antibodies	1	1.79
Autoantibody + mixture of alloantibodies	15	26.78
Autoantibody + unidentified antibodies	15	26.78
Autoantibody + mixture of alloantibodies + unidentified antibodies	10	17.86
Autoantibody + autoanti-I + unidentified antibodies	7	12.50
Autoantibody + autoanti-I + mixture of alloantibodies + unidentified antibodies	6	10.71
Total	56	100.00

Concerning 46 Rh negative and 5 weak D patients, only one D-negative male patient with history of transfusions developed anti-D. This patient received a single unit of Rh-positive blood under critical life-saving condition. This finding showed that either Rh negative or weak D patients have to receive only Rh-negative blood.[9]

The prevalence of red cell antibodies among patients at Thammasat University Hospital was 2.46%, similar to a related study at Taksin Hospital, Bangkok (2.62%)[14] but the prevalence was higher than that of patients in lower northern (0.54%)[15] and northeastern Thailand (1.89%).[16] Even though the positive antibody screening was more observed among females than in males, no significant difference was found in either sex. Moreover, the results of positive antibody screening tests among our patients showed significant associations with group B, resembling a related study conducting among the Thai patient and donor populations.[16,17]

As a result of antibody specificity, anti-Mi^a, anti-E, anti-c and antibodies in the Lewis system were predominant in this study. Antigen-negative red cells according to those above-mentioned should be prepared in our stock inventory, particularly group O and B donors. Routinely, blood bank personnel in a general hospital could perform and identify antibody specificity while samples with undetermined antibody specificities will be sent to a reference laboratory.[18] In this study, only 56 (9.64%) samples were transferred to NBC-TRCS and the problematic majority was due to autoantibody combined with a mixture of alloantibodies and unidentified antibodies. Therefore, the complicated cases may require additional techniques involving enzyme, adsorption-elution, and extra panels to determine antibody specificities.[1,18]

Regarding our findings, approximately 60% of problematic cases received phenotype-matched donors; whereas, 13 of 56 patients needed to receive the least incompatible blood under the physician's decision and presented no signals of post-transfusion hemolytic reactions. The other 10 patients were decided to not to be transfused by the attending physician. Of those patients, a mixture of alloantibodies were anti-Mi^a, anti-E and anti-c, so corresponding antigen typing in blood donors was primarily required for Thai patients. However, frequencies of other red cell antigens varied in Thai populations resulting in the different opportunities to obtain the desired number of antigen-negative donor units.

Conclusion

We analyzed problems in pretransfusion testing among patients at Thammasat Univesity Hospital, which represented potentially clinically relevant data to red cell antibodies. The association with frequently observed alloantibodies is predictive of the appropriate management of antigen-negative donor units to ensure prompt and safe blood transfusions.

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