

Original Article

The Evaluation of Platelet Crossmatching in Alloimmunized Thrombocytopenic Patients

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Objective: To evaluate the results of platelet crossmatching with two different methods and the response of platelet transfusion in alloimmunized thrombocytopenic patients. **Materials and Methods:** Forty-four alloimmunized thrombocytopenic patients with refractory to random platelet transfusions were included in this study. Twenty patients' sera were crossmatched with 2,871 lymphocytes and platelets samples from healthy donors using lymphocytotoxicity test (LCT) and solid phase red cell adherence assay (SPRCA) methods to evaluate the concordance and discordance of negative and positive results. The achievement of platelet transfusion between crossmatched and random platelets was determined using a CCI calculation. A 1-hour CCI > 7,500 or 24-hours CCI > 4,500 are considered acceptable. **Results:** Platelet crossmatching using LCT, when compared with SPRCA was significantly correlated ($p < 0.001$) and demonstrated the 85% concordance and the 15% discordance results among 2,871 crossmatched samples. The satisfactory CCI at 1-hour and 24-hours posttransfusion of 69 crossmatched transfusion episodes were 51% and 38%, respectively which were statistically significant greater than 109 random platelet transfusion events (30% and 14%, respectively) at $p < 0.05$. In addition, the satisfactory CCI of the crossmatched platelet transfusion was associated with SPRCA which gave negative and positive results of 95% and 5%, respectively. **Conclusion:** Platelet crossmatching using SPRCA combined with the LCT technique is an effective selection method for compatible platelets for alloimmunized thrombocytopenic patients.

Keywords : ● Platelet crossmatching ● Alloimmunized thrombocytopenia ● CCI ● Platelet refractoriness
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Introduction

Platelet transfusion refractoriness (PTR) is the condition which post transfusion increment is lower than expected and causes shortened transfused platelets' survival in recipient's circulation system.

PTR is usually found in multitransfused patients. The definition of refractory to platelet transfusion is the corrected count increment (CCI) at 10 minutes to 1 hour that is less than $7,500/\mu\text{L}/\text{m}^2$ body surface area per number of transfused platelets and at 18-24 hours which is less than 4,500. It is inadequate with inspire of giving an adequate dose of platelets with at least two consequences after transfusion¹. The refractory of platelet transfusion can be caused by immunity and non-immunity causes. The immunity causes are antibodies against

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the ABO system and human leukocyte antigens (HLA) and or human platelet antigen (HPA) which are on the platelet surface membranes. Approximately 80% of refractory cases are due to non-immune causes such as sepsis, fever, splenomegaly, disseminated intravascular coagulation (DIC), hepatic sinusoidal obstruction syndrome - also termed hepatic veno-occlusive disease, graft-versus-host disease (GVHD), hemorrhages and medications such as quinidine, penicillin, sulfa drugs, heparin, diuretics and vancomycin due to drug induce thrombocytopenia². The compatible platelets can be identified using several methods of platelet cross-matching. This study aimed to evaluate the difference of platelet crossmatch result between the lymphocytotoxicity test (LCT) and the solid phase red cell adherence assay (SPRCA), and to compare the response of alloimmunized thrombocytopenic patients who received cross-matched and random platelets transfusions.

Materials and Methods

The present study was approved by the Research Ethics Committees of Siriraj Hospital, Mahidol University, Thailand. There were 44 alloimmunized thrombocytopenic patients and donor platelets obtained from The Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University. Patient sera, donor lymphocytes and platelets were collected between May 2012 and February 2013. There were 20 patient sera and 2,871 samples of ACD blood obtained from healthy donors at the end of blood donation and kept at 4°C overnight before being used for LCT crossmatching. The platelet concentrates that had identical unit numbers with the ACD blood samples obtained from the segment of platelet bags in a sterile condition and kept frozen at $-70 \pm 5^{\circ}\text{C}$ for at least 1 hour before being used for SPRCA crossmatching³. The success of platelet transfusion was evaluated based on the Corrected Count Increment (CCI) of 69 episodes of LCT compatible platelets transfused into 20 patients

and 109 episodes of ABO identical random platelets transfused into 24 patients then the Corrected Count Increment (CCI) was calculated as follow.⁴

CCI =

$$\frac{(\text{Post platelet count} - \text{Pre platelet count}) \times \text{Body surface area (m}^2\text{)}}{\text{Number of platelets transfused (10}^{11}\text{)}}$$

A 1-hour CCI > 7,500 or 24-hours CCI > 4,500 are considered as response group

The Pre and post-transfusion platelet count at 1-hour and 24-hours, patient characteristics and the characteristics of the platelet transfusion were obtained from the medical records of each patient.

Statistical analysis

The platelet response between the crossmatched and random platelet transfusion involved the different results of the two methods analyzed by Pearson's chi-square test and others; age, gender, diagnosis, previous transfusion and clinical condition analyzed by descriptive statistics: mean, median, and percentage using SPSS version 16 software.

Results

Characterization of alloimmunized thrombocytopenic patients

All of the 44 alloimmunized thrombocytopenic patients included in this study had HLA reactive antibody tested by LCT and SPRCA techniques, 13 patients were male (30%) and 31 were female (70%) with a median age of 39 years (range from 16 to 81 years). They were separated into two groups of receiving crossmatching using LCT and SPRCA techniques and receiving random platelet transfusions. These patients had various diagnostic clinical complications from previous transfusions. More than 50% of them received medical and chemotherapy, only one patient in the random group had allogeneic hematopoietic stem cells transplant HSCT. (Table 1)

The results of platelet crossmatching

The total of 2,871 crossmatched samples of 20 alloimmunized thrombocytopenic patients tested by LCT and SPRCA methods showed the concordance of

Table 1 The characteristics of alloimmunized thrombocytopenic patients

Characteristics	Crossmatched platelet transfusion	Random platelet transfusion
	Case (%)	Case (%)
Patients	20	24
Age (median/range)	37 (16 to 81)	40 (16 to 81)
Gender		
Female	15 (75.0)	16 (67%)
Male	5 (25.0)	8 (33%)
Diagnosis		
AML	7 (35.0)	9 (37.5)
ALL	1 (5.0)	1 (4.2)
Aplastic anemia	1 (5.0)	2 (8.3)
SLE	4 (20.0)	4 (16.7)
Graves disease	2 (10.0)	2 (8.3)
Other malignancy	5 (25.0)	6 (25.0)
Previous transfusion (mean $\pm$ SD)	990 (49.5 $\pm$ 34.39)	1149 (47.9 $\pm$ 31.77)
Platelet transfusion	359 (18 $\pm$ 9.57)	414 (17.3 $\pm$ 8.89)
Other component	631 (31.5 $\pm$ 28.87)	735 (30.6 $\pm$ 26.67)
Gestational history	3 (15.0)	3 (12.5)
The clinical condition of patient		
Transfusion reaction	11 (55.0)	12 (50.0)
Fever	13 (65.0)	17 (70.8)
Infection	19 (95.0)	23 (95.8)
Sepsis	11 (55.0)	13 (54.2)
Bleeding	11 (55.0)	13 (54.2)
DIC	7 (35.0)	7 (29.2)
Splenomegaly	-	1 (4.2)
Splenectomy	1 (5.0)	1 (4.2)
Use of medication	13 (65.0)	16 (66.6)
Allogeneic hematopoietic stem cell transplantation	-	1 (4.2)
Chemotherapy	10 (50.0)	13 (54.2)

negative (1,269 - 78%) and positive (1,171 - 94%) results. The results of the LCT and SPRCA techniques were satisfactory and significantly correlated with the test strength agreement of $K = 0.702$ at $p < 0.01$. However, there were 77(6%) and 354(22%) of discrepancies between the two methods. (Table 2) The transfusion outcome of 20 patients who received crossmatched platelet transfusion in this present study was separated in to response and non-response groups, with 13 patients (65%) and 7 patients (35%), respectively.

Among 20 patients who received crossmatched platelet transfusion, nine hundred and twenty cross-matched pairs in the response group had a good agreement between the two techniques, which was better than 7 patients in the non-response group with the test strength agreement of $K = 0.711$, $p < 0.001$ and 0.566, $p < 0.05$ respectively. (Table 3)

Three hundred and sixteen compatible platelets from the total of 1,645 LCT crossmatched pairs were transfused to 20 alloimmunized thrombocytopenic patients

Table 2 The results of platelet crossmatching with LCT and SPRCA techniques

LCT	SPRCA		Total	p-value
	Negative	Positive		
Negative	1,269 (78%)	354 (22%)	1,623 (57%)	< 0.01
Positive	77 (6%)	1,171 (94%)	1,248 (43%)	
Total	1,346 (47%)	1,525 (53%)	2,871 (100%)	

LCT = Lymphocytotoxicity test; SPRCA= Solid phase red cell adherence assay

Table 3 The response of crossmatched and random platelet transfusion

	Crossmatched platelet transfusion	Random platelet transfusion	p-value
	N (%)	N (%)	
Number of patient	20 (45)	24 (55)	
Number of transfusion episodes	69 (39)	109 (61)	
1-hour CCI			
Satisfactory	35 (51)	33 (30)	< 0.05
Unsatisfactory	34 (49)	76 (70)	
24-hour CCI			
Satisfactory	26 (38)	15 (14)	< 0.001
Unsatisfactory	43 (62)	94 (86)	

Table 4 The results of SPRCA crossmatching in response and non-response group

SPRCA result	Response group	Non-response group	p-value
	N (%)	N (%)	
Negative	162 (95)	55 (38)	< 0.001
Positive	8 (5)	91 (62)	
Total	170 (54)	146 (46)	

in 69 transfusion episodes. Only 35 episodes (170 units) gave good response to the transfusion (51%). When using SPRCA, 162 units gave negative results (95%-162 in 170) and the other 8 gave positive SPRCA (5%).

In the other group, 34 crossmatched transfusion episodes were non- responsive (49%) with 55 units (38%), and when using SPRCA, 91 units were positive (62%). However, the different results in SPRCA crossmatching was statistically significant between the response and non-response groups with $\chi^2 = 121.223$, $p < 0.001$. While, 33(30%) out of the 109 episodes of random platelet transfusions were responsible for the therapy. (Table 3, 4)

The response of transfusion was having a good correlation between the 1-hour and 24-hours post-transfusions with a test strength agreement of $K = 0.74$

at $p < 0.001$ and the success of the platelet transfusion at the 1-hour post transfusion point showed statistically significant differences between the crossmatch and random platelet transfusion with $\chi^2 = 7.484$, p -value < 0.05 and the 24-hours point were $\chi^2 = 13.636$ at $p < 0.001$.

Discussion

The compatible platelet donors can be identified by various crossmatch methods. The lymphocytotoxicity test (LCT) has been used universally for antibody screening and crossmatching in platelet transfusion since it was first used in 1964⁵. Nowadays, various technologies for alloantibody detection are applied to crossmatching^{6,7}.

In this study, we evaluated the results of platelet crossmatching between LCT using the standard conventional cell-based complement dependent cytotoxicity (CDC) compared with solid phase red cell adherence assay (SPRCA). The results of both techniques were concordance in both negative and positive results among 431 crossmatched pairs, with 18% and 82%, respectively, having discrepancies. However, the different results of these two techniques may have arisen from some limitation in each technique. The recent study of Buakaew J and Promwong C found the sensitivity and specificity of LCT and SPRCA were 84.21%/95.83% and 94.73%/100%, respectively⁸. This present study found a good agreement between the two techniques in the response group, greater than in non-response group. However, the association between SPRCA crossmatched results and platelet response were statistically significantly different between the response and non-response groups.

The study showed that 35 out of 69 episodes (51%) of LCT crossmatched compatible platelet transfusions responded to the transfusion at the 1-hour and reduced to 26(38%) at the 24-hours post-transfusion. (Table 3). However, the normal platelet increment at 1-hour after the transfusion returned to the baseline after 24-hours where the shortening of platelet survival may be associated with the clinical condition of the patients and the use of some antibiotics or non immune cause. In addition, most of inadequate increment at the 1-hour post-transfusion was due to alloimmunization¹. We found that most of the patients who were transfused with crossmatched and random platelets had clinical complications due to non-immune factors which were similar with the study of Novotny et al.⁹ Most of these patients had an infection, with around 55% having sepsis due to fungal infection and during an infection episode, the production of alloantibodies can be stimulated and cause the non-responding to the platelet transfusion.^{10,11}

In this study, seven patients in each group had DIC that was associated with septicemia, the most common clinical condition found and was further associated with a poor response to transfused platelets. However, the study of Slichter et al. found that DIC was not affected at either the 1-hour or 24-hours post transfusion platelet increment, but instead decreasing the time to the next platelet transfusion and the controlling of DIC may improve the platelet count after transfusion.^{1,2,12}

Moreover, 70% of refractory oncohematologic patients had these clinical conditions and received drugs, especially amphotericin B, which was associated with a decrease in post-transfusion increments at 1 hour and at 18 to 24 hours and also decrease the time to the next transfusion and these affected both the post-transfusion platelet increment and refractoriness.^{2,13,14} There was one case in the random group that had splenomegaly and a failure to platelet transfusion due to the increasing of platelet pooling in spleen from 40% in a normal spleen to 80%.¹⁵ One of each group had a previous history of splenectomy and represented the success of platelet recovery in both crossmatched and some episodes of uncrossmatched platelet transfusion and may be from the status of spleen which having the greatest effect on improving platelet increment.²

However, we found 35% of twenty alloimmunized thrombocytopenic patients had a refractory to cross-matched platelet transfusion and most patients were associated with an infection.^{10,16} While the recent study of Aline AF and TRAP study showed that 22% and 71% of alloimmunized subjects, respectively, had an inadequate response to crossmatched platelet transfusion associated with the clinical factors.^{16,17} In this study, six of seven refractory patients had history of two or more pregnancies and had history of medication in five of them (71.4%) which the condition of immuno-suppressed in oncohematology patients induced by the underlying disease and chemotherapy should be considered for the role in facilitating infection.¹⁴ (Table 1)

Both techniques used in this present study were different from the other methods by using different specific target cells, even though both of them were time consuming and required personnel skill. Nevertheless, both requires small amount of serum, economical and only requires a small budget. They are cheaper than other methods and suitable as alternative techniques use when platelet crossmatching and antibody screening are required^{18,19} especially in alloimmunized thrombocytopenic patients.

Conclusion

The compatible crossmatched donor platelets are still needed for patients who are alloimmunized against platelet antigens and are refractory to random platelet transfusion. The use of SPRCA and combine with LCT technique are effective methods for the selection of compatible platelets for the alloimmunized thrombocytopenic patients. In addition , both can be used to prevent the refractory to platelet transfusion for patients requiring platelet transfusion as well.

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การประเมินการทดสอบความเข้ากันได้ของเกล็ดเลือดในผู้ป่วยที่มีเกล็ดเลือดต่ำและมีแอนติบอดีร่วมด้วย

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วัตถุประสงค์ เพื่อประเมินผลการทดสอบความเข้ากันได้ (crossmatch) ของเกล็ดเลือด ด้วยวิธีการที่แตกต่างกันสองวิธี และประเมินการตอบสนองของเกล็ดเลือดในผู้ป่วยที่มีเกล็ดเลือดต่ำและมีแอนติบอดีร่วมด้วย **วัสดุและวิธีการ** ทำการศึกษาในผู้ป่วยเกล็ดเลือดต่ำที่มีแอนติบอดีร่วมด้วยและไม่ตอบสนองต่อเกล็ดเลือดจำนวน 44 ราย นำซีรัมผู้ป่วยจำนวน 20 รายมาทดสอบความเข้ากันได้กับตัวอย่าง lymphocytes และเกล็ดเลือดของผู้บริจาคจำนวน 2,871 ราย ด้วยวิธี Lymphocytotoxicity test (LCT) และ Solid phase red cell adherence assay (SPRCA) ประเมินผลการทดสอบความเข้ากันได้ โดยเปรียบเทียบความสำเร็จของการให้เกล็ดเลือดระหว่างกลุ่มที่ได้รับการทำ crossmatch และไม่ได้ทำ crossmatch (random platelets) โดยการคำนวณค่า Corrected count increment (CCI) คือ ค่า 1-hr CCI > 7,500 และ 24-hrs -CCI > 4,500 เป็นค่าที่ยอมรับได้ **ผลการศึกษา** ผลการทำ crossmatch ของทั้งสองวิธีมีความสอดคล้องกันอย่างมีนัยสำคัญที่ ($p < 0.001$) จากการทำ crossmatch ในตัวอย่างเลือดจำนวน 2,871 คู่ พบว่ามีตัวอย่างเลือด 2,440 คู่ (ร้อยละ 85) ที่ทั้งสองวิธีให้ผลสอดคล้องกัน และมี 431 คู่ (ร้อยละ 15) ที่ให้ผลไม่สอดคล้องกัน เมื่อคำนวณค่า 1-hr CCI และ 24-hrs CCI ของกลุ่มที่ได้รับ crossmatched platelets จำนวน 69 ครั้ง พบว่าค่า CCI ที่ยอมรับได้ คิดเป็นร้อยละ 51 และ 38 ตามลำดับ ซึ่งมากกว่ากลุ่มที่ได้รับ random platelets จำนวน 109 ค่า ซึ่งมีค่า CCI ที่ยอมรับได้ คิดเป็นร้อยละ 30 และ 14 ตามลำดับ อย่างมีนัยสำคัญ ($p < 0.05$) นอกจากนี้ ค่า CCI ที่ยอมรับได้ยังมีความสัมพันธ์กับผลการทำ crossmatch ด้วยวิธี SPRCA โดยร้อยละ 95 ให้ผลเป็นลบ และร้อยละ 5 ให้ผลบวก **สรุป** การทำ crossmatch เกล็ดเลือดโดยวิธี SPRCA ร่วมกับ LCT เป็นวิธีการที่มีประสิทธิภาพในการคัดเลือกเกล็ดเลือดที่เข้ากันได้สำหรับผู้ป่วยเกล็ดเลือดต่ำที่มีแอนติบอดีร่วมด้วย

Keywords : ● Platelet crossmatching ● Alloimmunized thrombocytopenia ● CCI ● Platelet refractoriness

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