Evaluation of the effectiveness of platelet crossmatching by the solid-phase red cell adherence assay in adult patients of a tertiary care hospital in Thailand: A retrospective study

Thunnakhon Sinwatcharaphirom | Kusuma Apisawes | Janejira Kittivorapart

Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Correspondence
Janejira Kittivorapart, Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
Email: Janejira.kii@mahidol.edu

Abstract

Background and Aims: Platelet transfusion refractoriness is well aware to be associated with poor clinical outcomes. Patients with the alloantibody causing refractoriness required cross-matched compatible products to improve the platelet number. This study aims to evaluate the effectiveness and availability of platelet crossmatching provided by the solid-phase red cell adherence (SPRCA) technique in the context of a tertiary university hospital.

Methods: A retrospective chart review was performed of the records of 214 patients with platelet refractoriness in Siriraj Hospital, a tertiary university hospital in Thailand, between January 1, 2017, and December 31, 2020.

Results: The SPRCA technique successfully provided cross-matched compatible platelets to 114 patients (69.7%). Platelet crossmatching significantly improved the platelet counts, as shown by the increased 1- and 24-h corrected-count increments (p< 0.0001). No acute transfusion reactions were observed in these patients. Of the 114 patients who received cross-matched platelets, 82 patients (71.9%) survived at 30-day posttransfusion; whereas, 16 patients (14.0%) died within 7-day posttransfusion.

Conclusion: The SPRCA method can provide a high availability rate of cross-matched platelets, which is effective at stopping and preventing clinical bleeding conditions. This method is appropriate to apply for platelet crossmatching in the context of a hospital blood bank.

Keywords
corrected-count increment, hospital blood bank, mortality rate, platelet transfusion refractoriness, SPRCA
1 | INTRODUCTION

Platelet transfusion is a pivotal therapy for a patient who has thrombocytopenic bleeding. However, patients who receive multiple blood transfusions, for instance, those with hematologic or solid organ malignancies, or those who undergo stem cell transplantation, usually have a high risk of platelet refractoriness. Platelet refractoriness is a clinical condition defined as the failure to achieve a satisfactory platelet count after receiving a proper dose of a platelet product. There is evidence that platelet refractoriness is significantly related to poor clinical outcomes, for example, increased risk of bleeding, prolonged length of hospitalization, increased healthcare costs, and a decreased survival rate of patients. The etiologies of platelet refractoriness can be classified as immune and nonimmune causes. Previous studies observed that approximately 20% of clinical refractoriness cases were accounted for by the former cause. The developments of several alloantibodies have been reported to be the cause of this immunological refractoriness, namely alloantibodies to the human leukocyte antigen class I (anti-HLA class I), the human platelet antigen, the glycoprotein IV, or CD36 (anti-NaK), as well as the anti-ABO blood group.

There are various ways to check whether a patient has platelet refractoriness. Typically, in our routine practice, we use the corrected-count increment (CCI) at 1 and 24 h after platelet transfusion to assess the efficiency of platelet transfusions. To provide platelets to refractoriness patients, platelet crossmatching is commonly used. The assay is simple, can be applied in a timely manner, and is relatively cost-effective compared to the use of HLA-matched selection products. The compatibility is defined as there being no in vitro reactivity between the donor’s platelets and the patient’s plasma.

In our center, when clinical refractoriness of platelet transfusion is suspected, a physician typically evaluates the patient’s 1-h CCI. Then, the clinical team contacts the medical staff at our Department of Transfusion Medicine to consult on crossmatching platelet units. The medical staff will then evaluate if the clinical case fits with the immune cause of refractoriness or not. Once the request is approved, ethylenediaminetetraacetic acid plasma is sent to the HLA laboratory at our department for the SPRCA testing. Typically, the turnaround time for compatible platelets is between 6 and 24 h after receipt of the patient’s sample.

Currently, various methods of platelet compatibility testing are available. In 2017, we used the lymphocyte cytotoxicity test alternative with the solid-phase red cell adherence (SPRCA) technique for platelet crossmatching. We have adopted the SPRCA method for the test for all patients since 2018 due to the practicality and simplicity of the test, including the modest testing costs. However, to the best of our knowledge, no previous study has yet been done to evaluate the effectiveness of the SPRCA assay in terms of the clinical and laboratory outcomes in developing countries. Thus, this study aimed to determine the effectiveness and availability of platelet crossmatching provided by the SPRCA technique to in-patients with platelet refractoriness in a hospital blood bank in Thailand.

2 | MATERIALS AND METHODS

2.1 | Data source and study participants

After receiving approval from the Siriraj Institutional Review Board (COA no.195/2021), we retrospectively reviewed the data records of patients with platelet refractoriness in Siriraj Hospital, one of the tertiary care hospitals in Thailand, between January 1, 2017, and December 31, 2020. All patients were enrolled in this study if they requested platelet crossmatching by the SPRCA method. Patients were eligible if they were in the in-patient department, aged >15 years old, and were proved to have platelet refractoriness or had a history of platelet refractoriness by using the CCI formula (see the formula below). The exclusion criteria included: (1) the patient was pregnant at the time of inclusion, (2) the 1-h CCI could not be followed, (3) the clinical bleeding in the first 24 h after platelet transfusion could not be followed, (4) the acute transfusion reaction could not be followed, and (5) the survival status in the first 30 days posttransfusion could not be followed.

From the medical records, we reviewed the patient’s demographic data, their clinical diagnoses and comorbid conditions, indications for platelet transfusion, events of bleeding or a transfusion adverse event in the first 24 h after the platelet transfusion, and the 7- and 30-day survival rates (as shown in Table 1). For patients who died within 30 days after receiving the cross-matched platelets, we assessed and identified the causes of death to validate the association with thrombocytopenic bleeding.

2.2 | Study variables and measures

2.2.1 | Platelet products

The platelet products transfused to the patients in this study comprised two types: (1) single donor platelet (SDP) obtained from an apheresis donor, or (2) pooled platelet products prepared from pooled 4-unit buffy coats from whole blood donors, called leucocyte-poor pooled platelet concentrate (LPPC). Both products were verified to contain platelets at >3 × 10^11/unit. The SDP is a leukoreduction product (white blood cells <1 × 10^6/unit), whereas the LPPC is not. The platelet products were issued to the patients based on several parameters, for example, the availability of the stock, the matched ABO blood group, and the patients’ healthcare scheme.

2.2.2 | Corrected-count increment

The patients’ information, including weight, height, and platelet counts pre- and posttransfusion, were used to calculate the 1- and 24-h CCI according to the following formula:

\[
\text{CCI} = \left( \frac{\text{posttransfusion platelet count (10^9/L)} - \text{pretransfusion platelet count (10^9/L)}}{\text{platelet dose transfused (10}^{11} \text{)}} \right) \times \frac{\text{body surface area (m}^2\text{)}}{\text{patient weight (kg)}}
\]
We then compared the CCI values before and after the compatible cross-match platelet transfusion. The 1- and 24-h CCI values were used to determine the clinical refractoriness before performing an SPRCA crossmatch. If the 1-h CCI was >7500 and the 24-h CCI was >4500, it could be concluded that the patients had no clinical sign of platelet refractoriness. Certainly, a low value of 1-h CCI reflects that the cause of platelet refractoriness is mostly immune-associated, while if the 1-h CCI is normal, but the 24-h CCI is low, the cause is frequently nonimmune associated.

### 2.2.3 | SPRCA assay

The SPRCA method was performed using a Capture-P® Test Kit (Immucor Inc.) on an automated analyzer Galileo Neo (Immucor Inc.). In brief, donor platelets were prepared into platelet-rich plasma in a concentration of 80,000–150,000/µl from a 6 ml citrate phosphate dextrose adenine blood sample. A total of 22 platelet samples were loaded into the equipment along with the positive and negative controls. The plasma of a patient was centrifuged at 900 g for 10 min, aliquot, and added to the equipment. The crossmatching process was fully automated and was described in the manufacturer instruction. In brief, the platelets were washed and added to the Capture-P well at 50 µl/well in a 1:1 ratio with the patient's plasma. After 40-min incubation and two washes to remove the unbound antibody, the Capture-P® Indicator Red Cells were added and the mixture was centrifuged at 600 g for 2 min for result interpretation. Positive tests show adherence of indicator cells to the platelet-bound antibodies in part or all of the well bottom, depending on the reaction's strength. In the negative tests, the indicator red cells pellet down and pack in the center of the well bottom.

### 2.3 | Statistical analysis

All the statistical analyses in this study were calculated using SPSS Statistics version 18 (SPSS Inc.). We used descriptive statistics to describe the demographic and clinical data. The normality of the data distribution was tested using the Shapiro–Wilk test. The average of the skewed data was described by the median and interquartile range. The Wilcoxon signed-rank test was applied to compare the CCI before and after compatible-platelet transfusion. p<0.05 on the two-sided test was considered as showing statistical significance.

### 3 | RESULTS

In total, 214 patients requested cross-matched platelets. According to our selection criteria, we excluded 50 patients: 11 patients were in the outpatient department, 10 patients only requested platelet antibody screening and so did not receive cross-matched platelets, 26 patients were under 15 years old, and 1 patient was referred to another hospital before 30 days of treatment according to his healthcare scheme. Two patients were excluded due to unavailable data records. Thus, only 164 of the 214 patients considered passed the inclusion and exclusion criteria. We reviewed their histories

### Table 1: Demographic characteristics and underlying disease of study participants

| Parameters                                      | Patients enrolled in this study (n = 164) |
|------------------------------------------------|------------------------------------------|
| Sex                                            |                                          |
| Male                                           | 83 (50.6%)                               |
| Female                                         | 81 (49.4%)                               |
| Age (years)                                    |                                          |
| 15–34                                          | 35 (21.3%)                               |
| 35–54                                          | 52 (31.7%)                               |
| 55–74                                          | 62 (37.8%)                               |
| >75                                            | 15 (9.1%)                                |
| History of blood transfusion (units)            |                                          |
| 3–5                                            | 5                                        |
| 6–10                                           | 10                                       |
| 11–20                                          | 28                                       |
| >20                                            | 121                                      |
| Underlying disease                             |                                          |
| Hematologic disorders                          | 134 (81.7%)                              |
| Aplastic anemia                                | 16                                       |
| Myelodysplastic syndrome                       | 9                                        |
| Acute myeloid leukemia                         | 41                                       |
| Acute lymphocytic leukemia                     | 12                                       |
| Lymphoma                                       | 26                                       |
| Multiple myeloma                               | 13                                       |
| Chronic myeloid leukemia                       | 5                                        |
| Other hematologic conditions                   | 12                                       |
| Solid organ malignancies                       | 7 (4.3%)                                 |
| Others                                         | 23 (14.0%)                               |
| Indication of requested platelet               |                                          |
| Therapeutic                                    | 23 (14.0%)                               |
| Prophylaxis                                    | 141 (86.0%)                              |
| Blood group (n = 163)                          |                                          |
| O                                              | 64 (39.2%)                               |
| A                                              | 39 (23.9%)                               |
| B                                              | 50 (30.7%)                               |
| AB                                             | 10 (6.1%)                                |
| Number of compatible-platelet requests (units), median (range) | 2 (1–4) |
regarding blood and blood components transfusion that the patients had received before a diagnosis of platelet refractoriness. We observed that 121 patients (73.7%) had previously received more than 20 units of red blood cells and platelets, 28 patients (17.1%) had received between 11 and 20 units, 10 patients (6.1%) had received between 6 and 10 units, and 5 patients (3.0%) had received less than 5 units in their lives.

The CCI values were calculated based on available data. After the normality testing, we found that all the CCI values were not in the normal distribution. The 1-h CCI based on the 124 data records available at the time of the diagnosis of platelet refractoriness was 4049.67 (1594.25–7528.81)/μl (median and interquartile range); whereas, the 24-h CCI based on 85 data records was −1481.37 (−4848.68–1671.54)/μl. After receiving cross-matched platelets, the patients achieved better 1- and 24-h CCI values: 10069.36 (4334.08–18683.36)/μl and 3424.26 (−74.43–8613)/μl, respectively. The rising platelet counts from pre- to posttransfusion of cross-matched compatible platelets for both the 1- and 24-h CCI values were statistically significant (p< 0.0001) (see Figure 1).

Of all the 164 patients with platelet refractoriness, the SPRCA technique successfully provided cross-matched compatible platelets to 114 patients (69.5%). While focusing on clinical information, the majority of the patients (n = 134, 81.7%) had underlying hematological disorders. Note that two of the patients were diagnosed with Evans syndrome and immune thrombocytopenia, that is, two conditions of autoimmune platelet destruction. Seven patients suffered from solid organ malignancies and 23 patients had other medical conditions.

There were 17 patients (10.4%) who had active bleeding and required platelet transfusion for therapeutic purposes. Of these, the SPRCA method was able to provide compatible platelets in 16 patients. No patients had a new site of bleeding after receiving the platelets. All of these pre-existing bleeding events were not critical and could be monitored (Table 2). There was no acute transfusion reaction observed after the cross-matched compatible platelet transfusion.

In the first 24 h after receiving compatible platelets, 98 patients (85.2%) had no clinical bleeding. Among the 17 bleeding patients who required platelets for therapeutic purposes, 9 (7.9%) had a minimal mucosal and gastrointestinal hemorrhage, 4 (3.5%) had skin petechiae and superficial ecchymoses, 3 (2.6%) had haematuria, and 1 (0.9%) had vaginal bleeding. All these hemorrhages were pre-existing conditions that the patients had before receiving the cross-matched compatible platelets.

Of the 114 patients who received cross-matched platelets, 82 patients (71.9%) survived at 30-day posttransfusion; whereas, 16 patients (14.0%) died within 7-day posttransfusion. The major

| TABLE 2 Availability and outcomes of cross-matched platelet transfusions |
|---------------------------------------------------------------|
| Available compatible platelets by SPRCA method | n = 164 |
| Yes | 114 (69.5%) |
| No | 50 (30.5%) |
| Patients | n = 114 |
| 30 days survival (%) | 82 (72.0%) |
| 7 days mortality (%) | 16 (14.0%) |
| • Sepsis | 14 (12.2%) |
| • Lower gastrointestinal bleeding | 1 (0.9%) |
| • Pulmonary leukemic infiltration | 1 (0.9%) |
| 8–30 days mortality (%) | 16 (14.0%) |
| • Sepsis | 11 (9.6%) |
| • Hemorrhagic shock | 2 (1.8%) |
| • Upper gastrointestinal bleeding | 1 (0.9%) |
| • Pulmonary embolism | 1 (0.9%) |
| • Acute kidney injury | 1 (0.9%) |
| Bleeding sites at the time of the requested platelets | 17 (14.9%) |
| Gastrointestinal bleeding | 9 (7.9%) |
| Petechiae and ecchymoses | 4 (3.5%) |
| Haematuria | 3 (2.6%) |
| Vaginal bleeding | 1 (0.9%) |
| Acute transfusion-associated reactions | 0 (0.0%) |

Abbreviation: SPRCA, solid-phase red cell adherence.

FIGURE 1 Platelet count increment at 1- and 24-h pretransfusion compared with the 1- and 24-h posttransfusion CCI after receiving SPRCA cross-matched platelets. The posttransfusion CCI of both 1 and 24-h is significantly increased from the pretransfusion CCI (p< 0.0001). CCI, corrected-count increment; SPRCA, solid-phase red cell adherence.
cause of death in the latter group was sepsis (14 patients, 12.2%). One patient died from lower gastrointestinal bleeding (his last platelet count was 3000/μl), and another from pulmonary leukemic infiltration. A total of 16 patients (14.0%) died between 8 and 30 days after a cross-matched compatible platelet transfusion. Sepsis was also the principal cause of death in this group (11 patients, 9.6%). One patient was suspected to have pulmonary embolism and another one died from acute kidney injury. Three of the patients died from exsanguination. The last platelet counts of these patients were 10,000/μl, 10,000/μl, and 99,000/μl. One of them had intractable thrombocytopenic bleeding from dengue shock syndrome. Thus, the mortality rate at 30-day postplatelet transfusion was 28% and only four (3.5%) patients died from the hemorrhagic condition.

4 | DISCUSSION

The characteristics of the 164 patients recorded as having platelet refractoriness were investigated in this study. The majority (134, 81.7%) of these patients had underlying hematological diseases. The prevalence of platelet refractoriness among hematological patients in our study was higher than reported previously.10,11 The difference in the prevalence between our study and others was potential because: (1) the red blood cells and platelet products provided to these patients might not always have been leukodepleted products,12 or (2) there was an increased awareness of platelet transfusion refractoriness in the patients taken care of by hematologists. In addition, there was a trend of association between the numbers of previously transfused platelet units and the rates of platelet transfusion refractoriness (as demonstrated in Table 1). This reflected the tendency to increase antibody alloimmunization in heavily transfused patients, such as in hematological disorder patients.

The value of 1-h CCI before compatible platelet transfusion was 4049.67 (1594.25–7528.81)/μl which was lower than the cutoff value of 7500 and confirmed the refractoriness to random transfused platelets. The post-SPRCA cross-matched platelets improved this refractoriness with a posttransfusion 1-h CCI of 10069.36 (4334.08–18683.36)/μl (p < 0.0001). This finding is in agreement with the previous studies.10,13–15 We also found a significant increase in the 24-h CCI (p < 0.0001) when comparing the CCI pre- and posttransfusion. In this study, we observed that the decrease in the 1-h CCI was associated with platelet antibodies. Thus, SPRCA was found to be an effective method to provide compatible platelets for 114 of the 164 patients (69.5%) who requested the cross-matched platelets. Among these 114 patients, 62.3% successfully raised their 1-h CCI to over 7500.

To assess the clinical outcomes of the cross-matched platelets provided by the SPRCA method, we observed the clinical bleeding conditions in the first 24 h and the 7- and 30-day-mortality rates after the cross-match-compatible platelet transfusions. Clinical bleeding conditions are the most anticipated outcomes for thrombocytopenic bleeding. To the best of our knowledge, the current study is the first study evaluating both the laboratory and clinical outcomes of SPRCA cross-matched platelets in Thailand. We found that SPRCA could provide platelets to 94.1% (16 of 17 patients) who required cross-matched platelets to treat their concurrent bleeding. The time used to prepare SPRCA for the patients ranges from 6 to 24 h.

All the patients receiving cross-matched platelet did not need the second dose of platelet transfusion. No new bleeding events were noted in the first 24 h after the platelet transfusion and none of the patients had an acute transfusion reaction. Thus, our results highly suggest that platelet crossmatching performed by the SPRCA method can raise the satisfactory posttransfusion platelet count and improve the clinical features against immune-causing platelet transfusion refractoriness. Also, this correlated with the data from previous studies showing that platelet crossmatching provided proper platelet counts that could treat thrombocytopenia and prevent the clinical bleeding conditions in platelet transfusion refractoriness patients.7,11,15,16

Furthermore, we then evaluated the 7- and 30-day mortality rates of the patients transfused with cross-matched platelets. The survival rates were 86% (98 patients) and 72% (82 patients), respectively. Among the 32 deceased patients, fatal thrombocytopenic hemorrhage was identified in three of them. One patient died from a massive gastrointestinal hemorrhage with a normal platelet count. These results suggest that the transfusion of cross-matched platelets provided by the SPRCA method has a high 30-day survival rate and is recognizably safe to be used in platelet transfusion refractoriness patients.

This study has some limitations to note. First, there were incomplete and unavailable data in the records due to the retrospective nature of the study. Second, we did not have a control group to compare if the rates of alloimmunization were statistically greater in patients receiving higher numbers of blood units. This trend, however, implies that to prevent alloimmunization and platelet refractoriness, all patients should be given the blood or blood products transfusion only when there is a proper indication for transfusion. Additionally, in patients who were heavily transfused or who tend to have multiple transfusions in the future, leucodepleted products should be given to prevent the development of platelet transfusion refractoriness.

5 | CONCLUSION

Platelet crossmatching provided by the SPRCA method can provide a high availability rate of cross-matched platelets which is effective at stopping and preventing clinical bleeding conditions. The patients receiving platelets demonstrated a high 30-day survival rate. This method is appropriate to apply for platelet crossmatching in the context of a hospital blood bank.

AUTHOR CONTRIBUTIONS

Janejira Kittivorapart: Conceptualization and writing – review and editing. Thunnakhon Sinwatcharaphirom and Kusama Apisawes: Data curation. Thunnakhon Sinwatcharaphirom: Investigation and formal
analysis and writing – original draft preparation. All authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. Janejira Kittivorapart had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT
Janejira Kittivorapart affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID
Janejira Kittivorapart http://orcid.org/0000-0002-4051-8686

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