

## Transfusion of ABO-Incompatible HLA-Matched Platelets as Support for Patients with Acute Myeloid Leukaemia Undergoing Chemotherapy

Satoshi Yamasaki<sup>1,2\*</sup>, Junko Fujimoto<sup>2</sup>, Kentaro Kohno<sup>1</sup>, Masanori Kadowaki<sup>1</sup>, Sachiko Matsunaga<sup>2</sup>, Ken Takase<sup>1,2</sup> and Seiichi Okamura<sup>1,2</sup>

<sup>1</sup>Department of Hematology, Clinical Research Institute, Japan

<sup>2</sup>Blood Transfusion Service, National Hospital Organization, Kyushu Medical Center, Fukuoka, Japan

**Corresponding author:** Satoshi Yamasaki, M.D., Ph.D, Department of Hematology, National Hospital Organization, Kyushu Medical Center, 1-8-1-Jigohama, Chuo-ku, Fukuoka 810-8563, Japan, Tel: +81-92-852-0700; Fax: +81-92-847-8802; E-mail: [yamas009@kyumed.jp](mailto:yamas009@kyumed.jp)

**Received date:** Dec 16, 2015, **Accepted date:** Mar 24, 2016, **Publication date:** Mar 28, 2016

**Copyright:** © 2016 Yamasaki S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

**Background:** ABO-incompatible human leukocyte antigen (HLA)-matched platelets are used to manage thrombocytopenic patients with anti-HLA-A and/or anti-HLA-B (HLA-A/B) antibodies when ABO-identical donors are not available. This study assessed the effectiveness of ABO-incompatible HLA-matched platelets in an unselected group of patients with acute myeloid leukaemia (AML) undergoing cytotoxic chemotherapy.

**Material and Methods:** The study cohort consisted of 12 AML patients undergoing cytotoxic chemotherapy and administered HLA-matched single donor platelet transfusions. Patients positive for anti-HLA-A/B antibodies were defined as candidates for HLA-matched platelet transfusion. The effectiveness of platelet transfusions was determined by measuring corrected count increments (CCIs).

**Results:** The 12 patients received a total of 128 HLA-matched platelet transfusions. The median CCIs 1 hour after ABO-minor and -major incompatible HLA-matched transfusions were 11.4 (range: 3.2-24.9) and 12.4 (range: 3-37), respectively. There were no significant differences in 1- and 24-hour CCIs among ABO-identical and ABO-minor and major incompatible transfusions.

**Discussion:** ABO-incompatible HLA-matched platelets are effective in supporting thrombocytopenic AML patient's positive for anti-HLA-A/B antibodies and undergoing cytotoxic chemotherapy.

**Keywords:** HLA-matched platelets; ABO-incompatible; Acute myeloid leukaemia

### Introduction

Refractoriness to transfused platelets is a difficult problem in thrombocytopenic patients requiring frequent platelet transfusions [1]. High percentages of patients with malignant haematological disorders and chemotherapy-induced bone marrow aplasia have been reported to become refractory to random donor platelet transfusions. The corrected count increment (CCI) is the most widely used surrogate marker for evaluating the responses of patients refractory to platelet transfusions. CCI is calculated using the formula: [post-transfusion platelet count ( $10^9/L$ ) - pre-transfusion platelet count ( $10^9/L$ )]  $\times$  [body surface area ( $m^2$ )] / [platelet dose transfused ( $10^{11}$ )]. Refractory patients are generally defined as those with a 1-hour CCI less than 7.5 or two CCIs below 5 within 24 hours after transfusion of ABO-compatible random donor platelets stored for < 72 hours [2]. Refractoriness may be due to clinical or patient-related factors, including fever, sepsis or splenomegaly; product-related factors or immunological causes. Immunological destruction of platelets, mediated by alloantibodies directed against antigens on platelets, is frequently the principal factor or an important contributor to platelet refractoriness [3]. Clinical platelet refractoriness has been found to correlate with allo-immunisation against human leucocyte antigen (HLA) and human platelet antigen (HPA) [4,5]. These correlations

provide the basis for blood product selection strategies in the management of refractory conditions [6]. These patients are usually managed by identifying anti-HLA/HPA antibodies in their sera and transfusing HLA-matched platelets [6,7]. This approach requires building a panel of thousands of HLA-typed potential aphaeresis donors. However, the number of available volunteer donors is limited. ABO-compatible platelet transfusions in non-oncologic patients have been associated with significantly better CCIs than ABO-major incompatible platelet transfusions [8]. To our knowledge, however, no previous study has analysed the effects on patients with acute myeloid leukaemia (AML) undergoing chemotherapy of the transfusion of ABO-incompatible HLA-matched platelets donated by healthy volunteers with anti-A/B antibody titres  $\leq 1:128$ .

This retrospective study was therefore designed to evaluate the clinical usefulness of ABO-incompatible HLA-matched platelets in AML patients refractory to randomly selected platelets. The ability of this strategy to effectively improve post-transfusion platelet counts, as determined by CCI, was tested in platelet-refractory patients with AML undergoing cytotoxic chemotherapy.

### Materials and Methods

A review of patient records identified 12 AML patients in our institution who became refractory to transfusions of randomly selected platelets between November 2010 and October 2014. These 12 patients included six with de novo AML and six with myelodysplastic

syndrome (MDS) leading to overt AML. Informed consent was provided by all patients before their blood was genotyped. This study was approved by the Institute's Research and Ethics committee.

During the study period, these 12 patients were transfused with HLA-matched platelets within 72 hours of collection. All platelet preparations were from donors aged 18 to 69 years, belonging to the registry of HLA typed apheresis donors, and were provided by the Japanese Red Cross Society Kyushu Block Blood Center. Male donors were aged 18 to 69 years and weighed at least 45 kg; female donors were aged 18 to 54 years and weighed at least 40 kg. Each donor had a haemoglobin concentration of 12 g/dL or higher and a platelet count of 150 to  $600 \times 10^9/L$ , with a maximum blood donation frequency of 24 times per year. The platelet preparations were obtained by apheresis of  $\leq 400$  mL of collected blood. Platelet concentrates were collected by platelet apheresis machines and suspended in autologous plasma.

AML patients were defined as refractory to transfusion if their 24-hour CCI was  $<5$  following two consecutive platelet transfusions [2] and if they were positive for anti-HLA-A and/or anti-HLA-B (HLA-A/B) antibodies while remaining negative for anti-HPA antibodies. Patients and donors were typed for HLA-A and HLA-B class I loci using WAKflow (Wakunaga Corp., Hiroshima, Japan) and screened for anti-platelet antibodies using an anti-HPA-MMPHA panel (Olympus, Tokyo, Japan), according to the manufacturer's instructions. Patients with clinically overt sepsis or splenomegaly were excluded. Selected patients, including those with recurrent non-haemolytic and allergic transfusion reactions, were pre-medicated with first-generation antihistamines, such as dexchlorpheniramine. Prior to transfusion, the platelets were irradiated with 15 Gy gamma rays to prevent transfusion-associated graft versus host disease. Diluted sera were incubated in glass tubes at room temperature with a 3% commercial suspension of red blood cells, followed by centrifugation of the samples at 3400 rpm for 15 seconds. Agglutination was considered positive if the red blood cells remained agglutinated after gentle shaking. The

highest dilution causing agglutination was assumed to represent IgM antibody titers. The patients' demographic and clinical characteristics, including age, sex, diagnosis, and the presence of splenomegaly, fever or infection at the time of transfusion, were recorded. Also recorded were the ABO and Rh D blood groups of recipients and donors; body weight and height of the recipients; recipient history of red blood cell (RBC) and platelet transfusions; time to detection of anti-HLA-A/B antibodies; and platelet counts before, and 1 and 24 hours after, platelet transfusions. Body surface area was calculated as described [9]. Transfusions of blood components and patient responses to previous platelet transfusions were also recorded. The relationship of response to HLA-matched transfusions of ABO-identical platelets and those with minor and major incompatibilities were assessed using chi-squared tests. All statistical analyses were performed using the statistical package Stata Version 13 (Stata Corporation, College Station, TX, USA).

## Results

The 12 patients received a total of 128 HLA-matched platelet transfusions. Clinical data of these patients and transfusions are summarized in Table 1. The 12 patients included three men and nine women, of median age 68.5 years (range: 56 to 78 years). All nine female patients had a history of pregnancy and childbirth. Median time from the first transfusion to the detection of anti-HLA-A/B antibodies was 4 months (range, 1 to 28 months). Patients received a median 10.5 (range, 2 to 30) HLA-matched platelet transfusions. Of these 128 HLA-matched platelet transfusions, 54 were ABO-identical, whereas 21 had minor and 53 had major incompatibilities. Median ratios of ABO-identical and minor and major incompatibilities per patient were 37.5% (range, 0 to 84%), 10.2% (range, 0 to 40%), and 46.7% (range, 0 to 100%), respectively. The differences in 1- and 24-hour post-transfusion CCIs among these three groups were not statistically significant (Table 2).

	Patients (n = 12)
Age, years, median (range)	68.5 (56-78)
Sex (male/female), n (%)	3/9 (25/75%)
Diagnosis, n (%) AML	6 (50%)
MDS overt AML	6 (50%)
Weight, Kg, median (range)	48.2 (35.0-63.8)
Height, cm, median (range)	150.1 (137.0-171.2)
BSA, m <sup>2</sup> , median (range)	1.46 (1.15-1.72)
Chemotherapy at platelet transfusion refractoriness, Idarubicin+Ara-C/CAG/HD-AraC, n (%)	3/5/4(25/42/33%)
No. of units previously transfused, median (range) RBC (random donor units) Platelets	24 (6-132) 155 (50-340)
Time to detection of anti-HLA Abs, months, median (range)	4 (1-28)
HLA-matched platelets	
No. of transfusions, median (range)	10.5 (2-30)

No. of units, median (range)	177.5 (25-490)
Platelet count ( $\times 10^{11}$ )/no. of transfusions, median (range)	3.2 (2.4-3.9)
No. of platelet units/no. of transfusions, median (range)	16.2 (12.1-19.6)
ABO compatibility status/no. of patients (%)	
ABO identical, median (range)	37.5% (0-84)
ABO minor incompatible, median (range)	10.2% (0-40)
ABO major incompatible, median (range)	46.7% (0-100)

Abbreviations: AML: Acute Myeloid Leukaemia; MDS: Myelodysplastic Syndrome; BSA: Body Surface Area; AraC: Cytarabine; CAG: Cytarabine+Aclarubicin +Granulocyte Colony-Stimulating Factor; HD-AraC, high-dose AraC; RBC: Red Blood Cell; HLA: Human Leucocyte Antigen; Abs: Antibodies; No.: Number.

**Table 1:** Patient demographic characteristics and details of platelet transfusion.

	Transfusions (n = 128)	ABO identical (n = 54)	ABO minor incompatible (n = 21)	ABO major incompatible (n = 53)	P
Pre-transfusion platelet count ( $\times 10^9/L$ ), median (range)	1.3 (0.2-1.9)	1.2 (0.2-1.9)	1.3 (0.2-1.8)	1.3 (0.3-1.9)	0.75
1-hour post-transfusion platelet count ( $\times 10^9/L$ ), median (range)	3.8 (1.2-8.5)	3.8 (1.2-8.1)	3.4 (1.5-7.2)	3.9 (1.3-8.5)	0.47
24-hour post-transfusion platelet count ( $\times 10^9/L$ ), median (range)	2.3 (0.3-6.1)	2.3 (0.3-6.1)	2.4 (0.5-3.9)	2.2 (0.5-6.1)	0.86
CCI at 1 hour post-transfusion, median (range)	13.1 (3.2-37.4)	13.1 (3.2-28.4)	11.4 (3.2-24.9)	12.4 (3-37)	0.89
CCI at 24 hours post-transfusion, median (range)	5.2 (0-28.8)	5.2 (0-17.2)	5.1 (0-16.3)	5.1 (0-29)	0.94

CCI: Corrected Count Increment

**Table 2:** Assessment of usefulness of HLA-matched platelets.

## Discussion

This study was performed to assess whether ABO-incompatible HLA-matched platelets could effectively improve post-transfusion platelet counts in AML patients undergoing cytotoxic chemotherapy and refractory to transfusion of randomly selected platelets. As various patient- and product-related factors have been found to influence the outcomes of platelet transfusions, we attempted to minimise these factors by strict study inclusion criteria and by transfusing HLA-matched platelets within 72 hours of collection. Although platelet transfusions were given to AML patients undergoing chemotherapy, we found that 1- and 24-hour post-transfusion CCIs did not differ significantly following the transfusion of ABO-identical platelets and those with minor and major ABO incompatibilities. The most likely mechanism of platelet destruction in patients with a 1-hour CCI less than 7.5 and/or a 24-hour CCI less than 5 was alloimmunisation to HLA and/or other platelet antigens. This may have been due to the selection of HLA-matched platelets donated by healthy volunteers with anti-A/B antibody titres  $\leq 1:128$  resulting from antibody specificities that do not necessarily mediate platelet destruction.

Previous studies comparing ABO-identical and ABO non-identical platelet transfusions in adults and children have yielded conflicting results [10,11]. In some reports, ABO-identical or -compatible platelet transfusions were associated with better results in patients with nonmalignant conditions [8,12]. However, the availability of HLA-

matched ABO-identical platelets is very limited in clinical practice, especially in emergency circumstances, such as after cytotoxic chemotherapy.

In contrast, only the presence of anti-HLA-A/B antibodies was a significant predictor of refractory responses to platelet transfusions. Although this finding requires confirmation, similar observations were made following the transfusion of lower titre anti-A and anti-B group O apheresis platelets when ABO-identical platelets were unavailable [13,14]. Because all female patients had a history of childbirth, risk factors associated with pregnancy and childbirth were not evaluated. Large cohort studies may be necessary to identify risk factors in patients receiving HLA-matched platelets.

## Conclusions

Our results suggest that blood banks and transfusion services should develop large donor pools to identify ABO-identical donors of HLA-matched platelets because transfusion of ABO-incompatible platelets has been associated with fatal haemolytic reactions, increased need for red cell transfusions and other adverse effects. However, ABO-incompatible HLA-matched platelets donated by healthy volunteers with anti-A/B antibody titres  $\leq 1:128$  may be suitable for transfusion into platelet-refractory AML patients undergoing chemotherapy. Studies in larger numbers of patients are required to confirm these findings.

## Acknowledgements

We thank the patients and clinical staff for their participation in the study. The authors also acknowledge the Clinical Research Institute, Kyushu Medical Hospital for their editorial support. We thank Yukari Kuroda (Japanese Red Cross Society Kyushu Block Blood Center) for helpful discussions.

## Authorship Contributions

S.Y. participated in design, data analysis, and manuscript preparation; J.K. and S.M. collected the data; K.K., M.K., K.T, and S.O. reviewed the manuscript.

## Disclosure of Conflict of Interest

The authors declare that there are no competing financial interests regarding this article.

## References

1. Hod E, Schwartz J (2008) Platelet transfusion refractoriness. *Br J Haematol* 142: 348-360.
2. Davis KB, Slichter SJ, Corash L (1999) Corrected count increment and percent platelet recovery as measures of posttransfusion platelet response: problems and a solution. *Transfusion* 39: 586-592.
3. Vassallo RR Jr (2007) New paradigms in the management of alloimmune refractoriness to platelet transfusions. *Curr Opin Hematol* 14: 655-663.
4. Seftel MD, Growe GH, Petraszko T, Benny WB, Le A, et al. (2004) Universal prestorage leukoreduction in Canada decreases platelet alloimmunization and refractoriness. *Blood* 103: 333-339.
5. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, et al. (2005) Factors affecting posttransfusion platelet increments, platelet refractoriness and platelet transfusion intervals in thrombocytopenic patients. *Blood* 105: 4106-4114.
6. Vassallo RR (2008) Changing paradigms in matched platelet support. *Transfusion* 48: 204-206.
7. Rebulla P (2005) A mini-review on platelet refractoriness. *Haematologica* 90: 247-253.
8. Pavenski K, Warkentin TE, Shen H, Liu Y, Heddle NM (2010) Posttransfusion platelet count increments after ABO-compatible versus ABO-incompatible platelet transfusions in noncancer patients: an observational study. *Transfusion* 50: 1552-1560.
9. Du Bois D, Du Bois EF (1989) A formula to estimate the approximate surface area if height and weight be known. *Nutrition* 5: 303-311.
10. Lozano M, Heddle N, Williamson LM, Wang G, AuBuchon JP, et al. (2010) Practices associated with ABO-incompatible platelet transfusions: a BEST Collaborative international survey. *Transfusion* 50: 1743-1748.
11. Julmy F, Ammann RA, Taleghani BM, Fontana S, Hirt A, et al. (2009) Transfusion efficacy of ABO major-mismatched platelets (PLTs) in children is inferior to that of ABO-identical PLTs. *Transfusion* 49: 21-33.
12. Marktel S, Napolitano S, Zino E, Cappelli B, Chiesa R, et al. (2010) Platelet transfusion refractoriness in highly immunized beta-thalassemia children undergoing stem cell transplantation. *Pediatr Transplant* 14: 393-401.
13. Cooling LL, Downs TA, Butch SH, Davenport RD (2008) Anti-A and anti-B titers in pooled group O platelets are comparable to apheresis platelets. *Transfusion* 48: 2106-2113.
14. Romphruk AV, Cheunta S, Pakoate L, Kumpeera P, Sripara P, et al. (2012) Preparation of single donor platelet with low antibody titers for all patients. *Transfus Apher Sci* 46: 125-128.