Journal of the Egyptian Society of Haematology & Research, Vol. 4, No. 1, March: 57-63, 2008

Original Article:
Flow Cytometric Platelet Cross-Matching to Predict Platelet Transfusion in Acute Leukemia

DOUAA SAYED, M.D.*; RANIA BAKRY, M.D.*; NAHLA Al-SHARKAWY, M.D.**; ASMAA ZAHRAN, M.Sc.* and MUHAMMED R. KHALAF, Ph.D.*

The Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University and the Department of Clinical Pathology, National Cancer Institute, Cairo University**, Egypt.*

ABSTRACT

Background: A great variety of patient and product related factors influence the outcome of platelet transfusions. Our study assessed the predictive value of a flow cytometric platelet cross match test for the outcome of HLA matched and unmatched platelet transfusions in patients with acute leukemia.

Materials and Methods: Thirty nine patients (26 adults and 13 children), were included in this study. Patients received 60 ABO and RH compatible platelet concentrates ranging from 1 to 4 per patient. We performed flowcytometric platelet cross-matching, HLA class I typing by SSP for patients and complement dependent cytotoxicity (CDC) for donors and screening of HLA class I antibodies by ELISA. Effectiveness of platelet transfusion was evaluated using the corrected count increment (CCI) which was calculated at 60 minutes and 18-24 hours post transfusion. Multivariate analysis was performed to detect which variable, can predict transfusion response more than others.

Results: Cross-matched platelet transfusions associated with good response in 48.6% of transfusion events in adults and 75% in children. The non-crossmatched platelet transfusions associated with poor response in 83.3% in adults and 100% in children. In the presence of clinical factors or HLA alloimmunization in adults, cross-matched platelets were associated with good response in 29.6% and 22.2% respectively. In children this occurred in 81.8% and 66.7% respectively. In presence or absence of HLA matching, flow cytometry platelet cross-matching was the most predictor for transfusion response.

Conclusions: Due to difficulties to find frequent HLA matched donors for acute leukemia patients, flow cytometric platelet cross-matching may provide the most useful way for selecting donors. It is useful even in the presence of alloimunization but only in children.

Key Words: *Flow cytometric platelet cross-matching – HLA alloimunization – Platelet refractoriness.*

Correspondence to: Dr. Douaa Sayed. Director of Flow Cytometry Lab-Clinical pathology Department-South Egypt Cancer Institute-Assiut University; Mail: Clinical pathology Department-South Egypt Cancer Institute-Assiut University. Assiut- Egypt; E-mail: Douaa_sayed@hotmail.com
Tel: +20106261987; Fax: +2/88/2348609

INTRODUCTION

Platelet transfusion is an essential part of the treatment of cancer, hematological malignancies, marrow failure, and hematopoietic stem cell transplantation **[1]**. However, some patients fail to receive the full benefit of platelet transfusions because they do not achieve the appropriate platelet count increment following transfusion **[2]**.

Refractoriness to platelet transfusions is often multifactorial **[3]**. It is most likely due to non-immune factors, although immune factors can sometimes be responsible **[1]**. Non-immune factors associated with decreased post-transfusion platelet count increments include clinical conditions such as splenomegaly, infection, disseminated intravascular coagulation (DIC), females with 2 or more pregnancies, bleeding, fever and use of heparin or amphotericin **[3-5]**.

Platelets express human leukocyte antigens (HLA)-A, HLA-B, and platelet specific antigen (human platelet antigens [HPA]). There is a strong association between the presence of HLA antibodies in the transfusion recipient and platelet refractoriness, but the relation between

platelet-specific antibodies and refractoriness is weaker **[5]**. Before the widespread use of leucocyte-reduced blood components to prevent alloimmunization, 45-70% of chronically transfused patients developed antibodies to HLA class I antigens **[6]**. Chronically transfused patients become alloimmunized to HPA less commonly. The proportion of patients with antibodies to HPA varies, but ranges from 2% to 17% **[7,8]**.

Two main strategies have been used to transfuse alloimmunized patients: Matching donor-recipient HLA antigens and cross-matching platelets. HLA-matching involves identifying the HLA type of the recipient and transfusing platelets from donors with matched antigens. HLA matching requires the availability of large numbers of HLA-typed donors. Alloimmunized patients are often transfused with platelets from donors that are only partially matched **[1]**. Transfusion of HLA-matched platelet, have shortcomings, with up to 20% of HLA-identical platelet transfusions failing to achieve the target platelet increment **[9]**.

A commonly used alternative to HLA-matched platelets is the transfusion of cross-match compatible platelets **[10,11]**. Cross-matching compatibility has been used for identification of candidate platelet donor and may be beneficial in patients in whom refractoriness is due to HPA, so the HLA-matched platelet transfusion has no value **[12]**.

Platelet cross matching by flow cytometry was addressed in many studies **[13,14]**. However, the predictive role of flow cytometric platelet cross-matching on transfusion response and its affection by the presence of clinical factors, HLA alloimmunization and transfusion of HLA-matched platelet were not fully evaluated.

The aim of this study is to assess the predictive value of flow cytometric platelet cross-matching for the outcome of ABO compatible platelet transfusion.

MATERIAL AND METHODS

This study was performed on 39 randomly selected patients with acute leukemia, including 18 female and 21 male, 26 adults and 13 children, all presented to South Egypt Cancer Institute from February 2006 to June 2008. They received 60 platelet transfusions (43 in adults and 17 in children) of ABO and RH compatible leuko-reduced platelet concentrates (PCs) ranging from 1 to 4 per patient. The mean value of transfused platelets was $5.03 \pm 1.13 \times 10^{11}$. PCs were collected by platelet apheresis using cell separator, COBE Spectra, version 7 (Cobe BCT Inc, USA).

In all transfusions, patients were monitored for the presence of splenomegaly, active bleeding, sepsis and fever greater than 38.5ºC. Effectiveness of platelet transfusion was evaluated using the post transfusion corrected count increment (CCI) which was calculated at 60 minutes and 18-24 hours post transfusion according to the formula:

CCI= (Post transfusion platelet count-pre transfusion platelet count $\times 10^9$ x body surface area)/ dose of platelets transfused $\times 10^{11}$ **[15]**.

A poor response was designated as CCI at 60 minutes and at 18-24 hours post-transfusion below 4,500-5,000 and 2,500 platelets per microliter respectively **[11]**.

Flow cytometric platelet cross-matching:

Platelet antibodies in plasma (or serum) were detected by incubating patients' plasma/ serum with donors' platelets. Serum was centrifuged for 3 minutes at 4000 rpm. Patients' serums were diluted 9:1 in 0.3% EDTA-PBS. Twenty µL of diluted patient's serum were incubated with 20µL of PC for 45 minutes at 37ºC in a water bath. EDTA-PBS (0.3%) was added to the tube and centrifugation at 1000 rpm for 10 minutes at room temperature and removal of the supernatant were done. Twenty µL of FITC conjugated antihuman IgG (Becton Dickinson, USA) and PE conjugated CD41 (Diaclone, France) were added. CD41 was used to confirm that we were analyzing platelet population, 95% of the gated cells must be positive for CD41. The samples were incubated for 45 minutes at room temperature to be ready for analysis by flow cytometry.

Positive control: Plasma containing anti HPA-Ia antibodies was treated as a patient sample and was thereafter diluted 1:2 (strong positive) and 1:20 (weak positive) in 0.3% EDTA- PBS.

Negative control: Negative control (donor serum) was treated as a patient sample.

Acquisition and analysis:

Samples analysis was done using FACS Caliber (Becton Dickinson, USA) flow cytometry. Five thousands events in an appropriate gate surrounding the platelet population were collected and data stored.

Fluorescence intensity (FI) from gated population in a histogram (FL1/ counts) with negative control as overlay geometric mean was used for fluorescence ratio (FR) calculation as shown in Fig. (1). The results were visually inspected on the FL1 histogram **[16]**.

FR= FI sample/ FI negative control.

Evaluation of the run:

FR was repeated if FR ≤0.6.

IgG anti platelet antibodies 1:2 positive controls should have FR >6.0.

IgG anti platelet antibodies 1:20 positive controls should have FR ≥1.7.

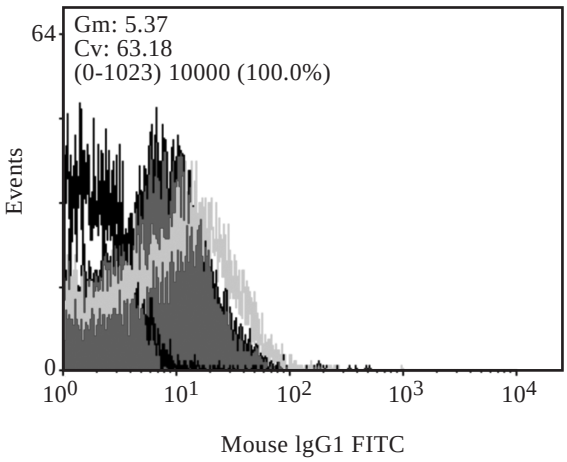

Fig. (1-A): A histogram showing a positive platelet cross-matching (red), negative control (black) and positive control (green).

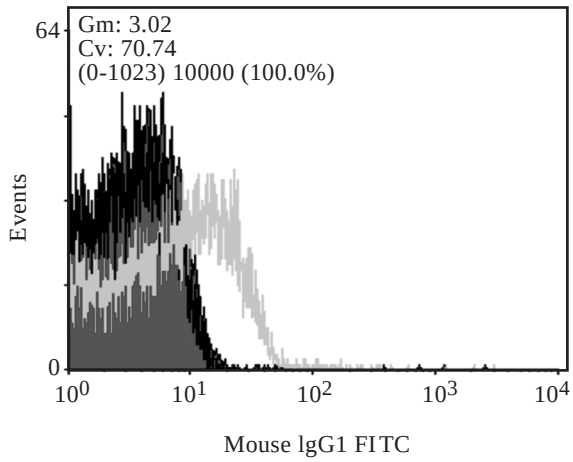

Fig. (1-B): A histogram showing negative platelet cross-matching (red), negative control (black) and positive control (green).

HLA class I typing by complement dependent cytotoxicity (CDC):

HLA class I typing for donors was performed by CDC method using HLA- ready plates ABC 72 (InnoTrain, Kronberg/ Taunus, Diagnostic GmbH).

SSP HLA class I A and B typing:

HLA class I typing for patients was performed by ALLSet+™ Gold SSP (Dynal Biotech Ltd., U.K). It is a PCR based technique, which uses SSP, for DNA based tissue typing. DNA extraction was done by GENE ALL+™ blood SV mini (General biosytsem, Korea).

PCR amplification was done according to manufacturer instructions. Amplicons were electrophoresed on 2% agarose gel. The assignment of alleles merely consists of determining whether amplification has occurred or not, i.e. visualization and detection of the amplification by agarose gel electrophoresis.

Detection of HLA class I antibodies:

Detection of HLA class I antibodies was done by ELISA using Lambda Antigen Tray (LAT^{TM}) (GmbH, Hannover, Grmany). ELISA was performed according to the manufacturer's instruction.

Statistical analysis:

Statistical package for social sciences (SPSS), version 15 was used for data analysis. Mean and standard deviation were used as descriptive value for quantitative data. Chi-square test was used to calculate relation between transfusion response and the presence of clinical factors, HLA alloimmunization in patients, platelet cross-matching and degree of HLA matching between patients and their donors. *p* value is significant when less than 0.05.

Receiver Operator Characteristic (ROC) curve was used for selection of the FR of platelet cross-matching which gives more specific

prediction of transfusion response. Logistic Regression analysis was done to detect which variable can predict transfusion response more than others. Association is present if the odd ratio is more than one. *p* value is significant when less than 0.05.

RESULTS

The clinical and laboratory data of all transfusion events are shown in Table (1). Good response to platelet transfusions was detected in 31 of the 60 transfusion events (51.7%), 19/43 in adults (44.2%) and 12/17 in children (71.3%).

Table (1): Clinical and laboratory data of 60 flowcytometric cross-matched platelet transfusion events.

Data	Adults (43)	Children (17)
Age	39±15.9	8±5.8
Male / Female	22/21	9/8
Splenomegaly	27 (62.8%)	13 (76.5%)
Bleeding	23 (53.5%)	7 (41.2%)
Fever	15 (34.9%)	9 (52.9%)
Infection	12 (27.9%)	5 (29.4%)
HLA antibodies before transfusion	20 (46.5%)	5 (29.4%)
Base line platelet count	17.25 ±12.32	15.69 ±9.28
Platelet count at one hour post transfusion	45.49±35.54	84.53±39.37
Platelet count at 24 hours post transfusion	31.52±26.38	57.35±30.96
Platelet dose (x 10^{11})	5.06±1.19	4.96±0.98
BSA of patients	1.66±0.15	0.87±0.42
CCI at one hour post transfusion	10.16±10.60	10.45±7.54
CCI at 24 hours post transfusion	5.58±6.78	5.98±4.39
Fluorescent ratio	1.33±0.62	1.2±0.31

CCI : Corrected count increment. BSA : Body surface area. HLA : Human leukocyte antigen.

The ROC curve was done to select the cutoff of FR of platelet cross-matching (Fig. 2). The *p* value of the ROC curve was 0.023. The FR less than 1.8 for negative platelet cross-matching (cross-matched platelets) gave the highest significance (p=0.017). So we considered it as the cutoff:

Negative= FR<1.8 Positive= FR≥1.8

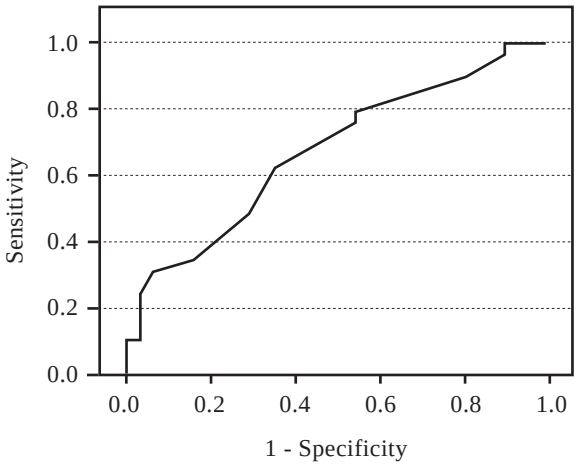

Fig. (2): The ROC curve for selection of the florescent ratio (FR) of platelet cross matching.

Predictive value of flow cytometric platelet cross-matching for platelet transfusion outcome:

Flow cytometric cross-matched platelets were transfused in 52/60 transfusion events (86.7%), 37/43 (86.1%) in adults and 15/17 (88.2%) in children. The difference between good and poor transfusion response regarding flow cytometric platelet cross-matching in the whole group was statistically significant. However, when the patients were divided into adults and children the difference was statistically significant only in the children group (Table 2).

Table (2): Role of flow cytometric platelet cross-matching in prediction of platelet transfusion outcome.

Transfusion response	Flow cytometric platelet cross-matching	
	Cross-matched platelets	Non cross-matched platelets
In all patients:		
Good	30/52 (57.7%)	1/8 (12.5%)
Poor	22/52 (42.3%)	7/8 (87.5%)
p value	0.017	
In Adults:		
Good	19/37 (51.4%)	1/6 (16.7%)
Poor	18/37 (48.6%)	5/6 (83.3%)
p value	0.143	
In Children:		
Good	11/15 (73.3%)	0/2 (0%)
Poor	4/15 (26.7%)	2/2 (100%)
p value	0.041	

p value was calculated by chi-square test

Influence of clinical factors on predictive value of flow cytometric platelet cross-matching:

Thirty eight (27 adults and 11 children) flowcytometric cross-matched platelet transfusions were given in the presence of and 14 (10 adults and 4 children) in the absence of clinical factors that might affect platelet transfusion outcome.

The difference in the predictive value of flow cytometric cross-matched platelets on transfusion response between patients with and without clinical factors is shown in Table (3).

Table (3): Influence of clinical factors on prediction of flow cytometric platelet transfusion response by platelet cross-matching.

Transfusion response	Flow cytometric cross-matched platelets	
	Presence of clinical factors	Absence of clinical factors
In all patients:		
Good	17/38 (44.7%)	12/14 (85.7%)
Poor	21/38 (55.3%)	2/14 (14.3%)
p value	0.008	
In Adults:		
Good	8/27 (29.6%)	9/10 (90%)
Poor	19/27 (70.4%)	1/10 (10%)
p value	0.001	
In Children:		
Good	9/11 (81.8%)	3/4 (75%)
Poor	2/11 (18.2%)	1/4 (25%)
p value	0.770	

p value was calculated by chi-square test

Influence of HLA alloimmunization on predictive value of flow cytometric platelet cross-matching:

Twenty one (18 adults and 3 children) flow-cytometric cross-matched platelet transfusions were given in the presence of and 31 (19 adults and 12 children) in the absence of HLA alloimmunization.

The difference in the predictive value of flow cytometric cross-matched platelets on transfusion response between patients with and without HLA alloimmunization is presented in Table (4).

Influence of using HLA matched platelets on predictive value of flow cytometric platelet cross-matching:

When HLA matched platelets (in 2 or more antigens) were used, cross-matched platelets transfusion was associated with good response in 10/11 (90.9%) while when transfused platelets were not HLA matched, the corresponding fig. was 7/13 (53.8%). The difference was found to be statistically significant ($p<0.05$).

Table (4): Influence of HLA alloimmunization on prediction of platelet transfusion outcome by platelet cross-matching.

Transfusion response	Flow cytometric cross-matched platelets	
	Presence ofHLA alloimmunization	Absence of HLA alloimmunization
In all patients:		
Good	6/21 (28.6%)	23/31 (74.2%)
Poor	15/21 (71.4%)	8/31 (25.8%)
p value	0.001	
In Adults:		
Good	4/18 (22.2%)	13/19 (68.4%)
Poor	14/18 (77.8%)	6/19 (31.6%)
p value	0.005	
In Children:		
Good	2/3 (66.7%)	10/12 (83.3%)
Poor	1/3 (33.3%)	2/12 (16.7%)
p value	0.519	

p value was calculated by chi-square test

Multivariate analysis:

Multivariate analysis was done to detect which variable (clinical factors, HLA alloimmunization and/or flow cytometric platelet cross-matching) can predict transfusion response. Flow cytometric platelet cross-matching was found to be the best predictor of transfusion response with the highest significance followed by HLA alloimmunization then clinical factors. HLA matching showed no predictive value (Table 5).

Table (5): Clinical factors, HLA alloimmunization, flow cytomtric platelet cross-matching and HLA matching as predictors for platelet transfusion response.

Variable	Flow cytometric platelet transfusion response	
	Odd ratio	*p*-value
Clinical factors	2.56	0.466
HLA alloimmunization	2.89	0.312
Platelet cross-matching	19.76	0.050
HLA matching	0.07	0.032

p value was calculated by Logistic Regression test

DISCUSSION

A great variety of patient and product related factors influence the outcome of platelet transfusions. The patient related factors are numerous, as weight, height, splenomegaly, fever, infection, DIC and HLA alloimmunization. Major platelet factors that are associated with poor response are decreased dose of platelets, ABO incompatibility **[17]** and platelets stored more than 48 hours **[18]**. In our study, we tried to minimize the influence of platelet factors by giving the patients ABO-RH compatible-leukoreduced PCs within hours of collection.

In this study flow cytometric platelet cross-matching was found to be a good predictor of transfusion response, transfusion of cross-matched platelets was more successful than transfusion of non cross-matched platelets. The significance of the area under the ROC curve is also indicating that flow cytometric platelet cross-matching is a good technique for platelet cross-matching. Rebulla et al. **[10]** reported the same result although they used another automated technique.

We noticed that flow cytometric platelet cross-matching is a better predictor of transfusion response in children than in adults. It had better prediction of transfusion response when clinical factors were absent than when clinical factors were present. In adults, the predictive role of cross-matched platelets on transfusion response is more affected by the presence of the clinical factors than in children. This reflects the importance of transfusion of flow cytometric cross-matched platelets especially after exclusion of the presence of the clinical factors in adults and even in the presence of clinical factors in children.

Many previous studies **[14,19-21]** stated that the ability of cross-matching to predict transfusion response may be lower in unselected patients with refractoriness to platelet transfusion than in those without associated clinical factors but their patients were with no specific age group.

The same results were observed when HLA alloimmunisation is absent. This better predictive value of platelet cross-matching for transfusion response after exclusion of clinical factors and HLA alloimmunization reflects the importance of transfusing cross-matched platelets to all patients especially in patients who have neither clinical factors nor HLA alloimmunization.

The best predictive role of flow cytometric platelet cross-matching in this study was when HLA matched platelets were used, transfusion of cross-matched platelet was associated with good response in 90.9% when platelets were matched in two or more HLA antigens. This Fig. is higher than that of Sintnicolaas and Löwenberg **[14]**, who found that when flow cytometric platelet cross-matching was negative, platelet transfusion was successful in 75% of transfusion episodes. This may be attributed, at least partially, to the different method used in evaluation of negative cross-matching. They expressed their results in percentage (negative platelet cross-matching less than 25%) while we used FR less than 1.8 as a cutoff for negative cross-matching. This may suggest that using the FR= 1.8 as a cutoff gives more accurate results.

By using multivariate analysis, flow cytometric platelet cross-matching was found to be the best predictor of transfusion response followed by clinical factors and HLA alloimmunization, while HLA matching had no predictive value. To the best of our knowledge, multivariate analysis was not done in previous studies. The lack of predictive value of HLA matching in our study can be explained by lower number of HLA matched donors, but this is not a defect in the present study only, because the finding of HLA matched donors is difficult due to the large number of polymorphisms in the HLA system that complicates the provision of HLA-matched platelets even if a large number of HLA-typed donors are available **[9]**. In a previous study **[22]** on 29 alloimmunized HLA-typed patients, the mean number of potential donors found in a file of 7247 HLA-typed donors, was 6 who were a four antigen match and 33 who were identical at two or three loci.

In addition, the techniques of HLA typing are time-consuming and costly. Also, it has been reported that approximately 40% to 50% of HLA-matched platelet transfusion events do not result in adequate increments **[23]**.

In conclusion, flow cytometric platelet cross-matching is the best predictor for transfusion response. It can be done on available apheresis

platelets, making a compatible transfusion available in few hours rather than the several days it takes to schedule, draw, and test an HLA-typed donor **[9]**. Potential donors, not identified by HLA matching, may be selected by cross-matching. This may be because such recipients have platelet antibodies rather than HLA alloantibodies and therefore would not respond to HLA-matched platelets **[23]**. This indicates the importance of transfusing of flow cytometric cross-matched platelets and that cross-matched platelets can be given to patients with acute leukemia from the start to predict the transfusion response. In children it may provide the most useful way for selecting donors even in presence of clinical factors and alloimmunization.

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