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Refractoriness to Platelet Transfusion After Single-Donor Consecutive Platelet Transfusions and its Relationship to Platelet Antibodies

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In thirty patients with acute leukemia and severe aplastic anemia receiving random single donor platelet transfusions, the development of refractoriness by consecutive platelet transfusions with cytapheresis and its relationship to the appearance of anti-platelet antibodies were investigated. The median number of platelet transfusions inducing refractoriness was 13 times, and 20% of the patients remained unrefractory despite of the repeated multiple platelet transfusions up to 20 to 25 times. The results of anti-platelet antibody tasts by the enzyme-linked immunosorbent assay(ELISA) and immunofluorescent techniques(IFT) showed no statistically significant relationship with the refractoriness($p > 0.1$). Although there was significant correlation between the results of ELISA and IFT, both tests were insufficient to find out refractoriness even with the use of pooled platelets from multiple donors as target cells. This study shows that 13 single donor platelet transfusions result in refractoriness, that both ELISA and IFT are insufficient to detect refractoriness despite of their significant correlation, and that other methods than these are needed in order to detect alloimmunization.

Key Words: *Refractoriness to platelet transfusion, anti-platelet antibody. Random single donor platelet transfusion*

INTRODUCTION

Prophylactic platelet transfusions have reduced the incidence of serious hemorrhagic complications in severely thrombocytopenic patients (Djerassi et al., 1963; Freireich, 1966; Cavins et al., 1968). However, repeated platelet transfusions cause the refractoriness, and thus decrease the effectiveness of further random donor platelet transfusions (Gmür et al., 1978; Dutcher et al., 1981a)

These refractoriness is the result of alloimmunization to human leukocyte antigen (HLA) and/or platelet specific antigen by the previous transfusions (Schiffer et al., 1976; Howard & Perkins, 1978; Pegels et al.; 1982).

The later finding is explained by the evidence that some of the patients with refractoriness still refractory to the platelet transfusion from HLA identical donor (Murphy & Waters, 1985). Thus not only the tets for HLA antiboies but also the tests for antiplatelet antibodies may be needed for the crossmatching and the detection of alloantibodies in platelet transfusions (Kakaiya et al., 1982).

Platelet pheresis which obtains platelet concentrates from random single donor is the major tools of prophylactic platelet transfusion in the management of thrombocytopenic patients. Thus we investigated to assess the characteristics of refractoriness development and to determine the number of platelet trans-

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fusions by which recipients become refractory to the consecutive random single donor platelet transfusions. In the observation of the relationship between the refractoriness and anti-platelet antibody, we performed anti-platelet anti-body tests with pooled platelets as target cells from 5 different donors. This was aimed to determine whether the results of these anti-platelet antibody tests are sufficient to find out the development of refractoriness.

MATERIALS AND METHODS

The characteristics of the patients are shown in Table 1. Total number of patients were 30, with 16 male and 14 female patients. Median age was 29 with range of 16 to 77 years. Eleven patients with acute nonlymphocytic leukemia, 9 with acute lymphocytic leukemia, 7 with aplastic anemia and 3 with blastic crisis of chronic myelogenous leukemia were entered. These patients had never received any blood components previously.

Donors, who satisfied the requirements of whole blood donation, had not ingested antiplatelet drugs for 5 days prior to donation, and had compatible ABO and Rh blood group with the recipients were eligible.

Platelets were harvested by differential centrifugation with intermittent flow (Model 30 S Haemonetics Corp., Braintree, MA) using trisodium citrate as anticoagulant, and transfused within an hour after harvest.

To determine the refractoriness, measurement of platelet count and corrected count increment (CCI) and tests of anti-platelet antibody were performed on each platelet transfusion.

Table 1. Characteristic of patients

No. of total patients		30
Male/Female		16/14
Age (year)	median	29
	range	16-77
ANLL		11
ALL		9
Aplastic anemia		7
CML blastic crisis		3

ANLL: Acute nonlymphocytic leukemia
ALL: Acute lymphocytic leukemia
CML: Chronic myelogenous leukemia

The refractoriness to platelet transfusion was defined as a posttransfusion corrected platelet count increment of less than 10,000 per μl per m^2 per 10^{11} platelets at 1 hour posttransfusion. CCI was calculated as:

$$\frac{(\text{Postcount-Precount}) \times \text{BSA in } m^2}{\text{Number of platelets transfused } (\times 10^{11})}$$

where BSA represents body surface area estimated from height and weight.

Platelet count was done with TOA PL-100 electronic platelet counter (TOA Medical Electronics Co., Ltd, Japan).

The enzyme-linked immunosorbent assay (ELISA) and immunofluorescence tests (IFT) were done after incubation of recipients serum with the pooled platelets from 5 different random donors of blood type 0

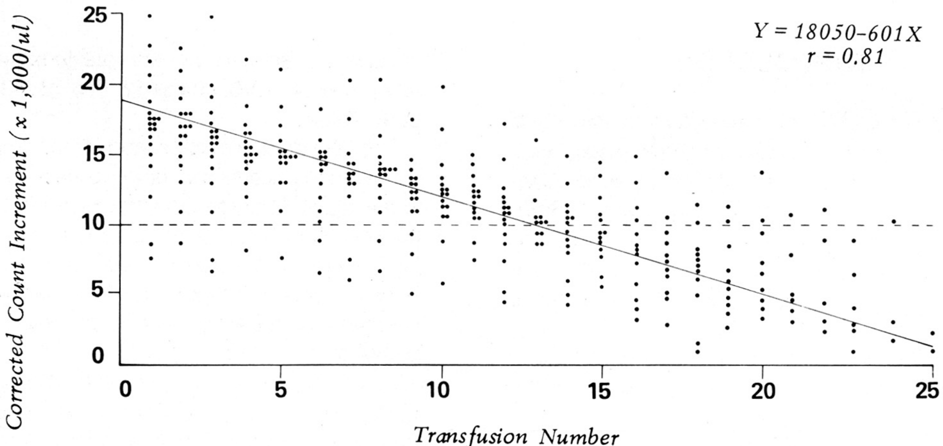

Fig. 1. Relationship between corrected count increment of platelet at 1 hour and transfusion number. On linear regression, after 13 times of platelet transfusions the corrected count increment became less than 10,000/μl and revealed the development of refractoriness.

Table 2. Characteristics of platelet concentrates

Volume(ml)	median	180
	range	130-240
Platelet concentration(/μl)	median	1.40×10^6
	range	0.99×10^6-2.34×10^6
Total platelet number(/pack)	median	2.53×10^{11}
	range	1.57×10^{11}-5.38×10^{11}
Leukocyte count(/μl)	median	4,200
	range	2,300-6,500
Red cell count(/μl)	median	3.9×10^4
	range	2.1×10^4-6.0×10^4

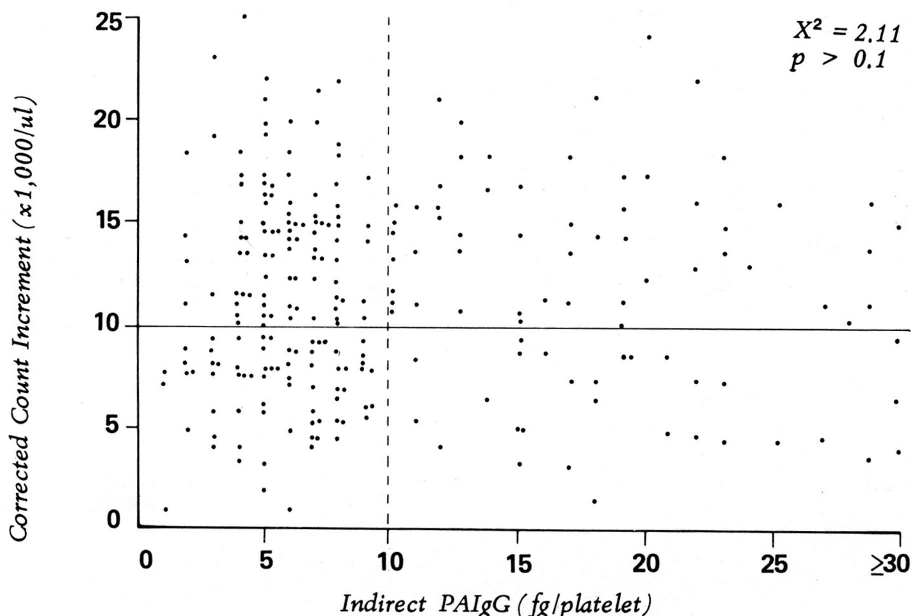

Fig. 2. Relationship between the result of enzyme-linked immunosorbent assay and the corrected count increment of platelet transfusion. The dotted line seperates negative (<10.0) from positive (≥10.0) results. Refractoriness to platelet transfusion is one in which the posttransfusion corrected count increment at 1 hour is <10,000/μl. PAIgG: Platelet associated immunoglobulin G

as target cells. Serum was obtained prior to each platelet transfusion.

In ELISA, amount of IgG antibody bound to platelets after incubation was estimated using peroxidase conjugated anti-human IgG as previously described (Park et al., 1987). A fluorescein isothiocyanate conjugated antihuman immunoglobulin (IgG+IgA+IgM) was used to estimate serum antiplatelet antibody in IFT as described elsewhere (Von dem Borne et al., 1978) with modification of using u-shaped 96-well microplate. Result was evaluated by a fluorescent microscope with epi-illumination (Nikon Co.).

The normal value of these tests were determined according to the results of healthy controls in our labolatory.

Linear regression was used to determine the number of platelet transfusions inducing refractoriness. Relationship between the results of both anti-platele antibody tests and between these results and CCI were analyzed by chi-square test.

RESULTS

The median number of platelet transfusions per patients during the study period was 14 times, which ranged from 5 to 25 times. The characteristics of plate-

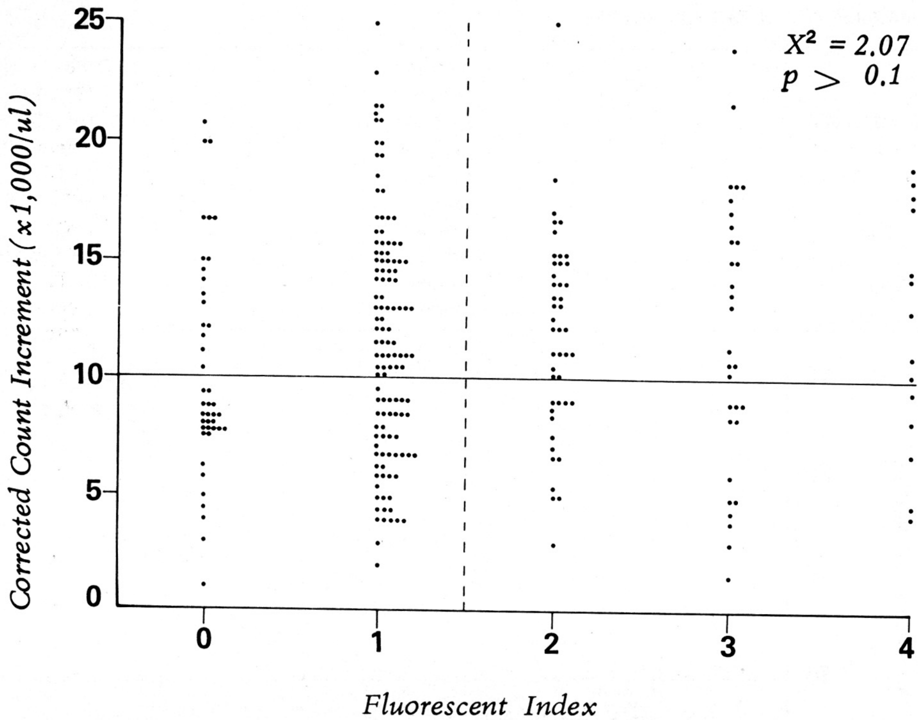

Fig. 3. Relationship between the indirect immunofluorescent antibody test and the corrected count increment. The dotted line seperates negative (0-1) from positive (2-4) results.

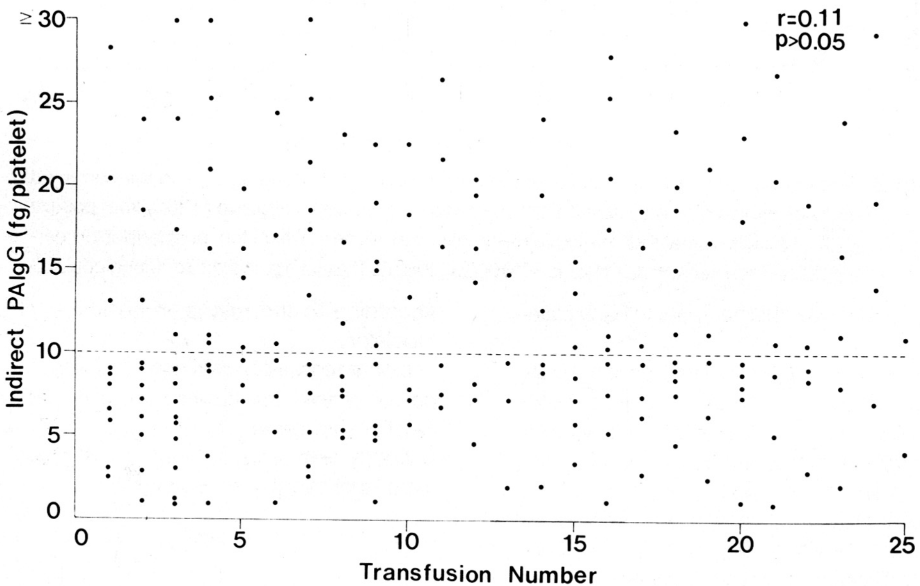

Fig. 4. Relationship between the results of enzyme-linked immunosorbent assay and transfusion number. The dotted line seperates negative (<10.0) from positive (≥10.0) results. PAIgG: Platelet associated immunoglobulin G

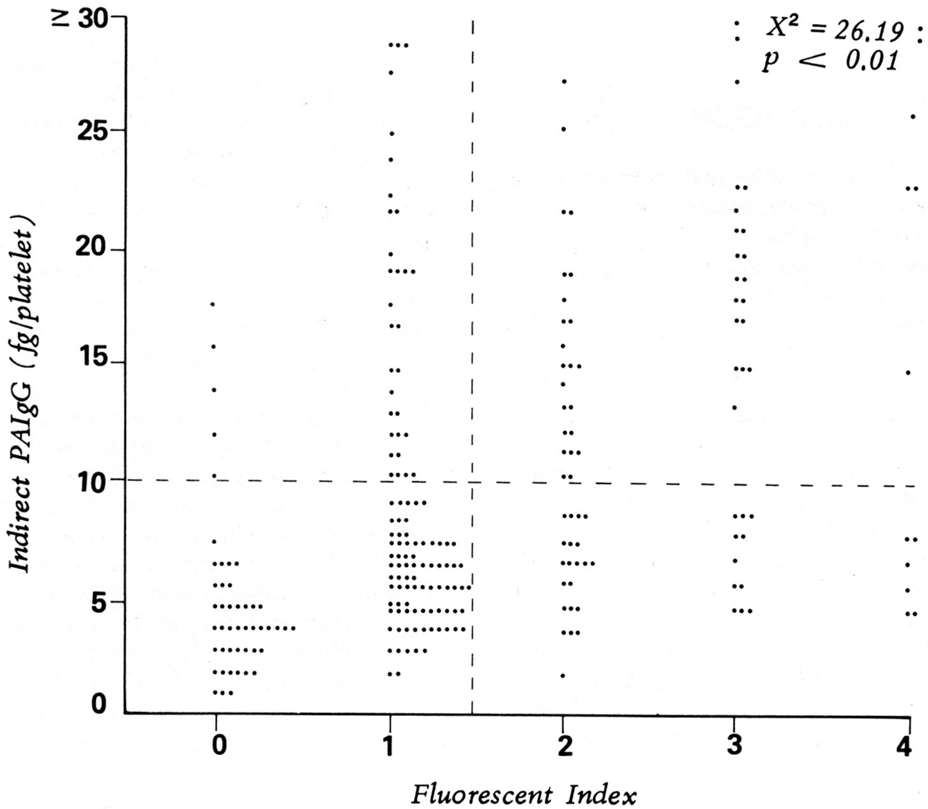

Fig. 5. Relationship between enzyme-linked immunosorbent assay and indirect immunofluorescent antibody test results. The dotted lines seperate negative from positive results of each test. PAIgG: Platelet associated immunoglobulin G

let concentrates are shown in Table 2. Median volume was 180 ml with the range of 130-240 ml, median plateled concentration was 1.4 million/μl (range 0.99-2.34 million/μl), and median platelet number per pack was 2.53×10^{11} which ranged from 1.57×10^{11} to 5.38×10^{11} Leukocyte contamination was 2,300-6,500/μl (median 4,200) and red cell contamination was 21,000-60,000/μl (median 39,000).

Trends of the refractoriness development were analyzed by comparing the CCI at 1 hour and the number of platelet transfusions (Fig. 1). The CCI showed progressive decrement with increase of the number of platelet transfusions, and on linear regression the equation of Y=18050-601X (r=0.81) was obtained. According to this equation, the CCI became less than 10,000/μl after 13 times of platelet transfusion, which reveals the median number of platelet transfusions inducing the development of refractoriness to the random single donor platelets. Twenty percent of the recipients remained without refractoriness even after 20 to 25 platelet transfusions.

Fig. 2 shows the relationship between the ELISA results and the CCI of the platelet transfusions. Fig. 3 shows the relationship between the IFT results and the CCI of platelet transfusions. The results of neither tests had statistically significant relationship to the CCI (X^2=2.11, p>0.1; X^2=2.07, p>0.1, respectively). Namely there was no notable appearance of anti-platelet antibody in the recipients, serum after the development of refractoriness and some patients had anti-platelet antibody even before the development of refractoriness. The positivity of ELISA and IFT was 23 and 24%, respectively. Nor there was significant relationship between the number of platelet transfusions and the results of anti-platelet antibody test (Fig. 4). But in the analysis to find out the concordance of those antibody tests, both anti-platelet antibody tests showed statistically significant correlation in their results (Fig. 5, X^2=26.19, p<0.01).

DISCUSSION

Platelet transfusion is the standard treatment for bleeding thrombocytopenic patient. The more recent acceptance of the prophylactic use of platelet transfusion for severe thrombocytopenia due to bone marrow failure has meant that patients with acute leukemia, aplastic anemia and those undergoing bone marrow transplantation often receive repeated platelet transfusions (Murphy & Waters, 1985). One of the major problems associated with multiple platelet transfusions from random donors is poor survival of the transfused platelets because of alloimmunization (Howard & Perkins, 1978; Grumet & Yankee, 1970), and this may become a severe complication in the management of these patients.

These refractoriness was defined by the CCI at 1 hour in our study, because the CCI at 4 or 24 hour frequently showed negative values in the patients who had platelet consumption factors such as fever, active bleeding and/or splenomegaly (data not shown). Our result showed that the median number of platelet transfusions inducing refractoriness was 13 times with random single donor platelet transfusion, whereas Gmur et al. (1983) reported that it was 6.3 times with multiple donor platelet transfusion. Therefore, in the prophylactic platelet transfusion, random single donor platelet is preferred to reduce or delay the development of refractoriness. However, Dutcher et al. (1981b) reported that the median number of platelet transfusions inducing refractoriness was not reached with random single donor platelets. This discrepancy may be explained by the relatively heavy leukocyte contamination in our single donor platelets, the use of other blood products with leukocyte contaminated, and the often repeated use of same donor in this study.

As other report (Howard & Perkins, 1978), 20% of our patients did not become refractory despite of repeated platelet transfusions. This is explained by the decreased immune responsiveness or the possible development of a state of relative immune tolerance in these patients (Dutcher et al., 1981b).

We have used ELISA and IFT as a potential technique for the detection of alloimmunization, and pooled platelets from 5 different donors were used as target cells. This was aimed to determine whether the results of these anti-platelet antibody tests are sufficient to find out the development of refractoriness by the reaction with multiple different HLA antigens in addition to the platelet-specific antigens. But the results of both tests had no significant correlation with the CCI, thus neither were sufficient to find out refractoriness. Whereas, it is well known that the usual indicator of alloimmunization is taken to be the presence of HLA antibodies (Murphy & Waters, 1985), and the incidence is in the range of 50-70% of patients receiving multiple platelet transfusions (Schiffer et al., 1976; Howard & Perkins, 1978; Pegels et al., 1982). Although immune response to platelet transfusion usually involve HLA antigens, platelet specific antigens are also important (Brand et al., 1978; Waters et al., 1981; Ware et al ., 1984). This finding explains the fact that the platelet transfusion with HLA-matched donor is not always successful in alloimmunized patients (Murphy & Waters, 1985). The frequency of platelet-specific antibody in immunized patients has been reported to be about 25% (Pegels et al., 1982), and our results also showed similar values. Because the results of both ELISA an IFT in this study had good concordance and adequate positive rates, the techniques themselves seem to be reasonable but inadequate to represent all of the refractoriness and/or alloimmunization. Thus it may be summarized that the ELISA and IFT is adequate to detect platelet-specific antibody, the use of pooled platelet from 5 different donors is insufficient to detect HLA alloantibody which is more prevalent and more responsible for the refractoiness, and these tests may be adequate to find out refractoriness if platelts from much more donors who have exclusively different HLA systems could be used as target cells.

There have been many reports concerning the prospective cross match techniques to select platelet donors for refractory patients. As earlier discussion, the use of HLA-matched platelet donors is not always successful in alloimmunized patients (Bucher et al., 1973; Tosato et al., 1978), suggesting that antibody against platelet-specific antigens is important in these cases. The best results of platelet cross-match have been obtained with platelet indirect antiglobulin techniques, often in association with a lymphocytotoxicity cross-match (Ware et al., 1984, Kickler et al., 1983). Kakaiya et al. (1984) reported that donor selection by lymphocytotoxicity compatibility did not appear to be useful if donors were selected by either ELISA or IFT crossmatch techniques. But in the view of both the detection of alloimmunization and the selection of compatible platelet donors, both HLA antibody and platelet-specific antibody have almost equal impor-

tance. Thus if platelet themselves or their antigens from approximately 50 different donors who have exclusively different HLA antigens could be obtained and preserved for a long time, it seems theoretically possible that the indirect anti-platelet antibody test using those platelets or antigens is fully sufficient to detect alloimmunization and to select compatible donors without the aid of lymphocytotoxicity test. But the feasibility and usefulness of this idea are left to be determined further.

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