Epitope-based matching for HLA-alloimmunized platelet refractoriness in patients with hematologic diseases

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BACKGROUND: For HLA-alloimmunized patients, platelet (PLT) concentrations are provided either at matched HLA-A and HLA-B loci or by serologic cross-reactivity groups (CREG) matching strategy. However, this method has some limitations.

STUDY DESIGN AND METHODS: In this study, the epitope-based matching (EBM) method was evaluated for selecting proper HLA-typed PLTs for patients with PLT transfusion refractoriness. Bead-based single-antigen HLA antibody detection method and HLAMatchmaker software were used to define the epitopes recognized by HLA-specific antibodies and to select compatible PLTs for nine patients with alloimmunized refractoriness. Corrected count increments (CCIs) were prospectively determined to compare successful transfusion rates among different matching methods in 142 PLT transfusions. In addition, HLA antibodies were serially detected to see whether any emerging antibodies appeared after receiving the EBM-matched PLTs.

RESULTS: The transfusion success rates evaluated with 1-hour CCIs for perfect matching or lacking any mismatching at HLA-A and -B locus (A/BU)-matched, CREG-matched, and EBM-matched PLTs were 85.2, 63.2, and 83.7%, respectively. Compared to CREG-matched PLTs, EBM-matched PLTs showed better transfusion results ($p = 0.035$). In the follow-up study (7 months; range, 3-13 months), no emerging HLA-specific antibodies were detected after receiving EBM-matched PLTs.

CONCLUSIONS: EBM performed on the basis of bead-based single-antigen HLA antibody detection coupled with the HLAMatchmaker program is recommended in choosing proper PLTs for refractory patients when A/BU-matched PLTs were not available.

Patients with hematologic diseases or thrombocytopenia require frequent platelet (PLT) transfusions to prevent or treat bleeding complications. Unfortunately, long-term PLT transfusions are complicated by refractoriness, which is defined as an insufficient PLT count increment after transfusion in patients receiving repeated PLT transfusions. PLT refractoriness is also presented secondary to coexisting nonimmune causes such as fever, splenomegaly, sepsis, disseminated intravascular coagulation, or medications.[1] However, immune refractoriness occurs most frequently in multiply transfused patients. To our knowledge, immunologically mediated unresponsiveness to PLT transfusions mainly results from the alloantibodies against human leukocyte antigen (HLA)-A and -B locus antigens and rarely from those against human PLT antigens. Therefore, it is recommended to provide HLA-matched or compatible PLTs for patients who have developed HLA antibodies.

ABBREVIATIONS: A/BU-matched = perfect matching or lacking any mismatching at HLA-A and -B locus; AML = acute myeloid leukemia; AUC = area under the receiver-operating characteristic curve; CREG(s) = cross-reactive group(s); EBM = epitope-based matching; MDS = myelodysplastic syndrome; ROC = receiver-operating characteristic.

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TRANSFUSION **;**:**-**.

Strategies for PLT selection for HLA-alloimmunized patients have been suggested by Hod and Schwartz:[2] 1) selecting HLA antigen–compatible PLTs is essential for ensuring good PLT response after transfusion, and 2) the PLTs selected should not have the antigens against which the patient has antibodies. However, if HLA-identical PLTs are unavailable, HLA-matched or HLA-partial-mismatched PLTs should be selected according to serologic cross-reactivity groups (CREGs) matching strategy.[3] CREGs are described as public epitopes that are differentially shared among HLA Class I molecules. HLA-A and HLA-B molecules can be grouped into nine or more families of CREG on the basis of serologic cross-reactivity patterns. This strategy has been implemented to decrease the size of the donor pool required to support all alloimmunized patients with matched products.

However, this approach is not effective enough; in a previous study, Moroff and colleagues[4] reported that the unsatisfactory increment rate was up to 40% of CREG-matched transfusions in patients with HLA intra-CREG antibodies. Owing to the limitations of CREG matching, a better tool is required for the selection of HLA-compatible PLTs.

For HLA-alloimmunized patients, the epitopes on HLA antigen should be more considered than HLA-specific antigens before PLT transfusion, because antibodies react with epitopes on antigenic molecules.[5] HLAMatchmaker is a structural matching algorithm that considers amino acid residue polymorphisms to define epitopes recognized by antibodies. This novel design could increase the number of donor candidates compatible for a given recipient.[6] The algorithm performs intra- and interlocus comparisons between the patient's and donor's HLA type and calculates the number of triplet amino acid or eplets sequence mismatches for HLA specificity. Although the effectiveness of HLA-matched PLTs transfusion using HLAMatchmaker software has been validated by many studies,[7,8] those studies only determined HLA compatibility by using the number of mismatched triplet amino acid or eplets but not defining the reactive epitope. There has been no report that demonstrates the effectiveness of HLA antigen or epitope avoidance mismatches in a long-term follow-up study of HLA-alloimmunized refractory patients. In this study, we identified the antibodies in patients by using the Luminex platform coupled with a highly sensitive antibody-binding assay and analyzed the antibody (or epitope) reactivity patterns with HLAMatchmaker. Finally, we used these predicted epitope patterns to determine the HLA compatibility for PLT transfusions in HLA-alloimmunized patients. We termed this epitope-based matching (EBM).

We prospectively evaluated PLT transfusion outcome in HLA-alloimmunized patients by using various matching methods including perfect matching or lacking any mismatching at HLA-A and -B locus (A/BU) matched, CREG matched, and the EBM method. The variables we evaluated were transfusion success rate and patient characteristics. We also evaluated additional HLA-specific antibodies that emerged during this prospective study period.

MATERIALS AND METHODS

Patients and study design

A random group of 73 patients with hematologic illnesses (33 females and 40 males) who had received multiple PLT transfusions at National Taiwan University Hospital (Taipei, Taiwan) were enrolled from September 2006 to May 2007. This study was approved by the institutional review board of the hospital (Serial Number 9561709011), and written informed consent was obtained from all patients. Pre- and posttransfusion PLT counts were analyzed with an automatic blood cell analyzer (Sysmex SE-2100, Sysmex, Kobe, Japan). HLA Class I and Class II antibodies were detected by Luminex mixed-antigens beads array (LifeScreen, Tepnel Lifecodes Corporation, Stamford, CT). Of 73 patients, 23 were identified as HLA-alloimmunized patients. Only 19 of these 23, who had a history of consistently poor increments at least twice after randomly selected PLT transfusions, had their PLT transfusion records reviewed. Although a total of 1111 PLT products were transfused to these 19 patients, before transfusion and 18 to 24 hours after transfusion, PLT counts necessary to calculate corrected count increment (CCI) values were available for only 183 (16%) transfusions. These 183 transfusions were evaluated retrospectively.

Of the 73 patients studied, nine patients were further prospectively evaluated from September 2006 through May 2008. The median duration of follow-up was 7 months (range, 3-13 months). There were three males and six females, with a median age of 64 and 57 years, respectively. For sequential study, blood samples were collected before and after 1 hour of PLT transfusion to perform PLT counts and HLA antibodies test. Clinical responses after PLT transfusion, including bleeding, fever, transfusion reaction, and medications, were recorded for the entire patients under study.

HLA antibodies were determined at first entering this study and at subsequent samplings every 2 months. Post-transfusion CCIs were used to monitor PLT transfusion efficiency. The CCI was calculated using the formula:

$$\mathrm{CCI} = (\text{post PLT count} - \text{pre PLT count} \times 10^9/\mathrm{L}) \times \text{body surface area}\,(\mathrm{m}^2)/\text{PLTs transfused} \times 10^{11}.$$

Refractoriness was defined as 1-hour CCIs of less than 7.5/L or 24-hour CCIs of less than 4.5/L after PLT transfusions.

During the sequential study, for providing the appropriate PLT concentrates, both patients and donors were molecularly HLA Class I typed by two commercial HLA SSO typing kits (Dynal Biotech Ltd, Bromborough, Wirral, UK; and Tepnel Lifecodes Corporation, respectively).

Donor-recipient pairing was done on the basis of conventional criteria.[9] First priority to PLT transfusion was A/BU-matched product. If A/BU-matched PLTs were not available, PLTs were selected by CREG or the EBM method through a prospective randomized trial design. Subsequent evaluation of clinical outcome and HLA antibodies in circulation after PLT transfusions were compared among these three matching methods.

HLA antibody screen and characterization

HLA antibodies were identified by using the Luminex mixed-antigens beads array platform. Assays were performed according to the manufacturer's protocol. The Luminex mixed-antigens assay–positive samples were further tested using single-antigen beads (Lab Screen Single Antigen Class I antibody detection system, One Lambda, Canoga Park, CA). The single antigen HLA antibody detection beads identified the following specificities: HLA-A1, 2, 3, 11, 23-26, 29-34, 36, 43, 66, 68, 69, 74, 80; HLA-B7, 8, 13, 18, 27, 35, 37-39, 41, 42, 44-65, 67, 71-73, 75-78, 81, 82; and HLA-Cw1, 2, 4-10, 12, 14-18. To determine if an individual bead was positive, fluorescence intensity values for the single-antigen beads reactions were calculated as the cutoff value for reactive samples by the manufacturer's software (HLA Fusion, One Lambda). The fluorescence intensity of each HLA-specific bead was normalized with the negative control beads and adjustment was made for the background fluorescence intensity caused by negative control serum, using the mathematical formula provided by the manufacturer. For the purposes of this study, mean fluorescence intensity (MFI) values less than 1255 after subtraction of background fluorescence intensity were considered negative.

Epitope prediction analysis of the detected HLA-specific antibodies using HLAMatchmaker software

HLA-A and HLA-B types of patients and donors were determined by low-resolution polymerase chain reaction and sequence-specific oligonucleotide probes method and each assigned to the most common corresponding four-digit HLA allele for our population. Amino acid sequences of the HLA antigens or alleles were downloaded from the IMGT/HLA Database. HLAMatchmaker programs and their instructions can be downloaded from the http://www.hlamatchmaker.net Web site.[10] The recipient's four-digit HLA allelic type was entered into the program. The resulting data of the HLA specific antibodies represented by the single-antigen beads were then entered into the program and eliminated the acceptable epitopes for determining the critical epitope mismatches for mismatched specificities.

EBM

When the critical epitope mismatches for each patient were identified we chose donors without the HLA antigens containing these reactive mismatch epitopes for the refractory patients. Because HLA single-antigen antibody detection by Luminex assay can detect very-low-titer antibodies, sometimes it is difficult to define the cutoff value of MFI for each reactive bead. We used the MFI value of donor-specific antigens from Luminex assay as a predictor for the PLT transfusion outcomes (successful response based on 18- to 24-hr CCIs), which were retrospectively collected from 183 PLT transfusion events in the 19 HLA-alloimmunized patients. To determine the operating feasibility of using MFI value as a predictor of successful response for PLT transfusion, we constructed the receiver-operating characteristic (ROC) curve. The area under the ROC curve (AUC) was 0.82 (Fig. 1). We used the MFI cutoff value of 1255 as a cut point for HLA antigen avoidance selection for highly HLA-alloimmunized refractory patients.

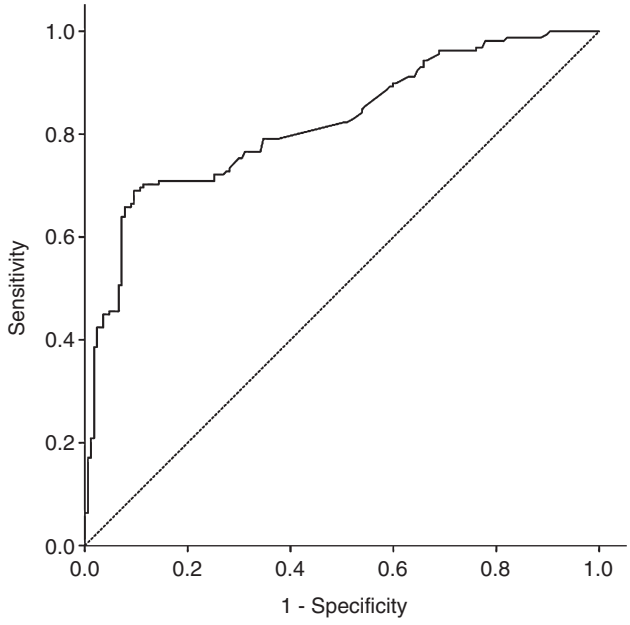

Fig. 1. ROC curve to measure accuracy of the MFI of donor-specific antibodies from HLA beads array data as PLT transfusion outcome predictor, using 183 transfusion results from 19 HLA-alloimmunized patients. ROC curves show (1 – specificity) versus sensitivity when using different thresholds of the MFI to predict transfusion outcomes (success or failure). The AUC is 0.82 ($p < 0.001$).

Statistical analysis

Descriptive statistics such as frequencies and percentages provided a general description of sample characteristics. The nonparametric Kruskal-Wallis test, chi-square analysis, or Fisher's exact test, as appropriate, were used to determine the levels of significance of differences between transfusion responses to PLTs of different matching methods. The ROC curve was constructed, and the AUC was calculated for fluorescence intensity of PLT transfusion outcomes. A two-tailed significance level of 0.05 was used in all statistical tests. All statistical analyses were performed using statistical software (SPSS, SPSS, Inc., Chicago, IL).

RESULTS

Patients' characteristics

Before enrollment in this study, HLA antibodies had been detected in 23 (31.5%) of the 73 patients. Their characteristics, transfusion history, and clinical treatments are shown in Table 1. There were no significant differences between HLA-alloimmunized and nonimmunized groups in terms of age, sex, red blood cells (RBCs) and PLT transfusion units, and various treatments. Among 67 patients who had HLA-A/B typing information, 26 (38.8%) patients were homozygous for HLA-A or/and -B demonstrating alloantibodies to HLA Class I locus occurred more frequently in the patients who were homozygous for HLA than those who were heterozygous (odds ratio = 4.45; confidence interval [CI] = 1.46-13.57; p = 0.007).

HLA antibody pattern in patients with alloantibodies

For the 23 HLA-alloimmunized patients with detected HLA antibodies, epitope analyses of reactive antibodies were performed by HLAMatchmaker and shown in Table 2. Antibodies were reactive with private and public epitopes on HLA antigens. Highly reactive eplets were 145QRT, 65QIA, 62QE, 127K, and 163EW. Only one private epitope 151AHA (A11) was found frequently. More antibodies against public epitopes than against private epitopes were found in multiply transfused patients. In addition to antibodies against public epitopes, intra-CREG antibodies were detected in 15 (65%) patients. Furthermore, 12 (53%) patients had anti-HLA-C that are rarely considered in routine PLT transfusion practice. Figure 2 shows the representative pattern of one patient (Case 21) with a sequential HLA-specific antibodies analysis during an 11-month study. Five critical epitopes (151AHA, 70IAQ, 177D, 71SA, and 44RT) of HLA antigens reacting with antibodies were identified in this patient. The antibody-reactive patterns of HLA antigens sharing the same epitopes were similar, demonstrating the accuracy of epitope analysis by HLAMatchmaker.

TABLE 1. Patient characteristics at study entry and in the HLA-alloimmunized group

Characteristics	Total (n = 73)	Non–HLA alloimmunized (n = 50)	HLA alloimmunized (n = 23)
Age (years), mean (SD)	48.81 (20)	46.34 (20)	54.17 (19)
Female, number (%)	33 (45.2)	21 (64)	12 (36)
HLA-A, HLA-B type,* number (%)			
Homozygous	26	12 (46)	14 (54)
Heterozygous	41	32 (78)	9 (22)
Transfusion history, median (range)			
RBCs†	30 (0-182)	28 (0-182)	60 (6-158)
PLTs†	58 (6-383)	37 (6-359)	52 (21-270)
Disease (%)‡			
AML	37	30 (81)	7 (19)
CML	5	3 (60)	2 (40)
ALL/CLL	5	4 (80)	1 (20)
MDS	9	3 (40)	6 (60)
SAA	12	6 (50)	6 (50)
Others	5	4 (80)	1 (20)
Treatment (%)§			
Chemotherapy	44	32 (73)	12 (27)
Immunosuppressive agents	16	11 (69)	5 (31)
BMT/SCT	12	9 (75)	3 (25)

* Only 67 of 73 patients were typed HLA-A or HLA-B locus. HLA homozygous includes one locus or both.
† RBCs transfusion includes whole blood, RBCs, and leukoreduced RBCs transfusion. PLT transfusion includes HLA-matched PLTs and random- or single-donor PLT transfusions.
‡ CML = chronic myeloid leukemia; ALL = acute lymphoblastic leukemia; CLL = chronic lymphoblastic leukemia; SAA = severe aplastic anemia; MDS = myelodysplastic syndrome.
§ Patients usually received antithymocyte globulin as immunosuppressive drug in our study group. BMT = bone marrow transplantation; SCT = stem cell transplantation.

TABLE 2. Epitope predictions of HLA-alloimmunized patients

Case*	Disease	Sex	Age (years)	HLA type	Reactive private epitope†	Reactive public epitope‡
1	AML	Male	62	A2,11 B13,75		65QIA/177DT(7C),44RE(8C)
2	AML	Male	68	A1,11 B37,60		62GE/127K(2C),163LW(5C),65QIA(7C),79RI(Bw4)
3	AML	Female	34	A2,26 B39,48	151AHA(A11),45RMA (B15)	62QE/144KR (1C),44RK (12C)
4	AML	Female	29	A11,11 B75,51		163EW/65QIA(7C)
5	AML	Female	61	A2,2 B51,60	151AHA(A11),76ESI (A32), 161D (A3)	62QE/90D(1C), 9T/145QRT(10C)
6	AML	Female	57	A24,24 B7,13	151AHA(A11),161D (A3)	62GE/145KHA(2C),9T/145QRT/193AV(10C)
7	CML	Male	48	A2,24 B60,60	151AHA(A11)	62QE(1C),71SA/44RT/163LW(5C),70IAQ(7C),62RN/145QRT(10C)
8	CML	Male	44	A2,26 B46,46		41T(12C),82LR(Bw4),80ERN(Bw6)
9	MDS	Male	64	A33,33 B58,58	151AHA(A11)	144KR/275EL(1C),127K/145KHA(2C), 71NT/45RMA(5C), 163EW(7C),145QRT(10C),80ERN(Bw6)
10	MDS	Male	89	A2,24 B51,54	12SMR(A6802)	90D/62QE(1C),9T/145QRT(10C)
11	MDS	Male	77	A2,2 B46,58	151AHA(A11)	144KR(1C),71NT(5C),65QIA(7C),62RN/145QRT(10C),56R(19C)
12	MDS	Male	71	A2,24 B46,60	151AHA(A11)	62QE(1C),71SA(5C),70IAQ(7C),145QRT(10C)
13	MDS	Male	71	A11,11 B60,60		167DG(1C),62GE/127K(2C),79RI(Bw4),167ES
14	MDS	Female	72	A33,33 B58,58	151AHA(A11)	62QE/144KR/167DG(1C),127K(2C), 71NT/45RMA(5C)163EW(7C), 145QRT(10C),80ERN(Bw6)
15	MDS	Female	75	A11,11 B46,60	76ESI(A32)	62GE/127K(2C),70IAQ(7C),9T/145QRT/193AV(10C),79RI(Bw4)
16	SAA	Male	47	A2,2 B13,46	151AHA(A11)	65QIA(7C),145QRT(10C)
17	SAA	Male	15	A2,2 B60,62		62QE/144KR(1C),65QIA(7C),9T/62RN/145QRT(10C),79RI(Bw4)
18	SAA	Female	40	A3,33 B35,58		65GKH (9C)
19	SAA	Female	29	A2,24 B46,46		71NT(5C),70IAQ/163EW(7C)
20	SAA	Female	23	A11,24 B61,62		44RT(5C),65QIA(7C),80IAQ(Bw4)§
21	SAA	Female	57	A2,2 B46,61	151AHA(A11)	44RT/71SA(5C),70IAQ/177D(7C)
22	other	Female	61	A2,2 B38,55	151AHA(A11)	45RMA/44RT(5C),163EW(7C)
23	other	Female	52	A2,11 B46,51		65QIA/177DT/163EW(7C),44RK(12C)

* Case numbers for follow-up study are underlined.

† Epitope prediction analysis was performed using HLAMatchmaker program and indicated by a single amino acid or residues within a 3-Å radius at the specific position on the HLA molecule. For example, 127K is a lysine residue in the polymorphic position 127.

‡ Nomenclature used for cross-reactive group (C) was based on the description by Rodey[18] (in parentheses). Antibodies reactive among the cross-reactive group excluding recipient's self-antigens are underlined.

§ 80IAQ (80I + 90A + 144Q) was a modified epitope from HLAMatchmaker eplets template and HLA epitopes study.[17]

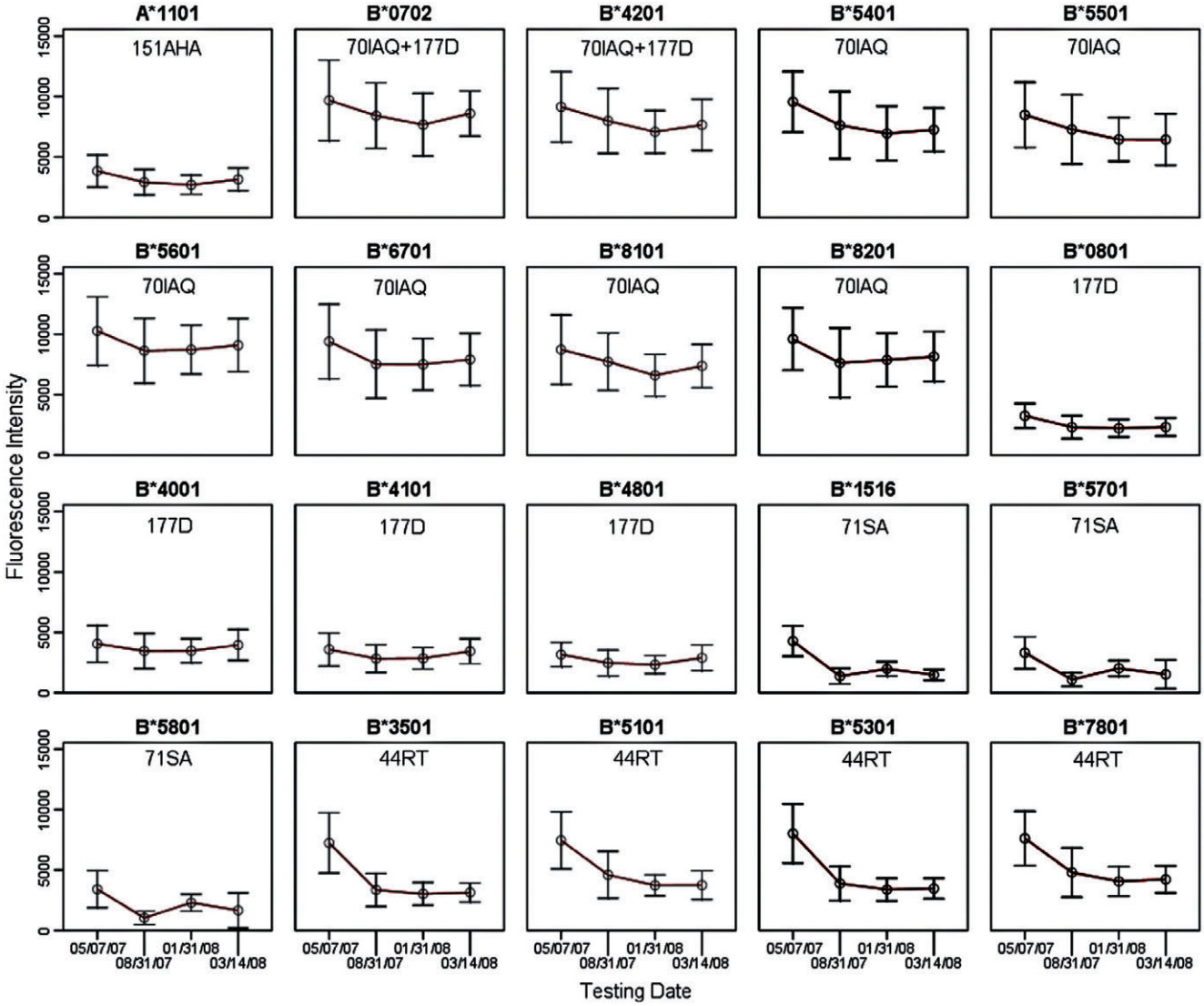

Fig. 2. Time-series reactive HLA Class I antibody patterns of Patient 21 using HLA single-antigen beads array assay for the 11-month follow-up study. Fluorescence intensity was indicated by the reactive antibody level. The error bars show mean ± 1 SD. The epitopes (e.g., 151AHA) shown in the figure are results from epitope prediction analysis by HLAMatchmaker. This patient was a 57-year-old female with severe aplastic anemia (HLA phenotype A2, 2; B46, 61).

HLA matching methods determined the PLT transfusion outcome

The 183 PLT transfusions collected retrospectively from 19 HLA-alloimmunized patients were analyzed for 24-hour CCIs evaluation. Of these, 23 were matched with the A/BU method, 29 with the CREG method, 29 with the EBM method, and others with random selection. The success rates of all kinds of HLA-matched PLTs were superior to randomly selected single-donor apheresis PLTs. However, there were no significant differences in the 24-hour CCIs and success rates (CCI > 4.5/L) among the A/BU-matched, CREG-matched, and EBM-matched PLT transfusions.

To properly evaluate various methods of PLT transfusion, nine patients were enrolled for the prospective study. The 1-hour CCIs of 142 single-donor PLT transfusions from these nine patients were evaluated. The median CCIs and success rates (CCI > 7.5/L) of each type of matched PLT are shown in Table 3. Regarding transfusion effectiveness, there were significant differences among using these three matching methods ($p = 0.021$). The EBM method gave results similar to those obtained by the A/BU method; however, they were better than the results obtained by the CREG method ($\chi^2 = 4.44$; $p = 0.035$). Overall, the decision-making order of matching method for HLA-alloimmunized patients

TABLE 3. Transfusions grouped by different HLA matching criteria

Method	A/BU matched	CREG matched	EBM matched	p value
N = 142	61	38	43	
Median CCI*	14.55 (10.38-22.17)	10.12 (2.11-26.32)	22.03 (9.85-30.87)	0.034†
Successful transfusions	52 (85.2%)	24 (63.2%)	36 (83.7%)	0.021‡

* Median CCI is presented with the first and third quartile values in parentheses.
† Kruskal-Wallis test.
‡ Chi-square test; there was significantly higher success rate of EBM-matched PLTs than CREG-matched PLTs (proportion test, p = 0.004).

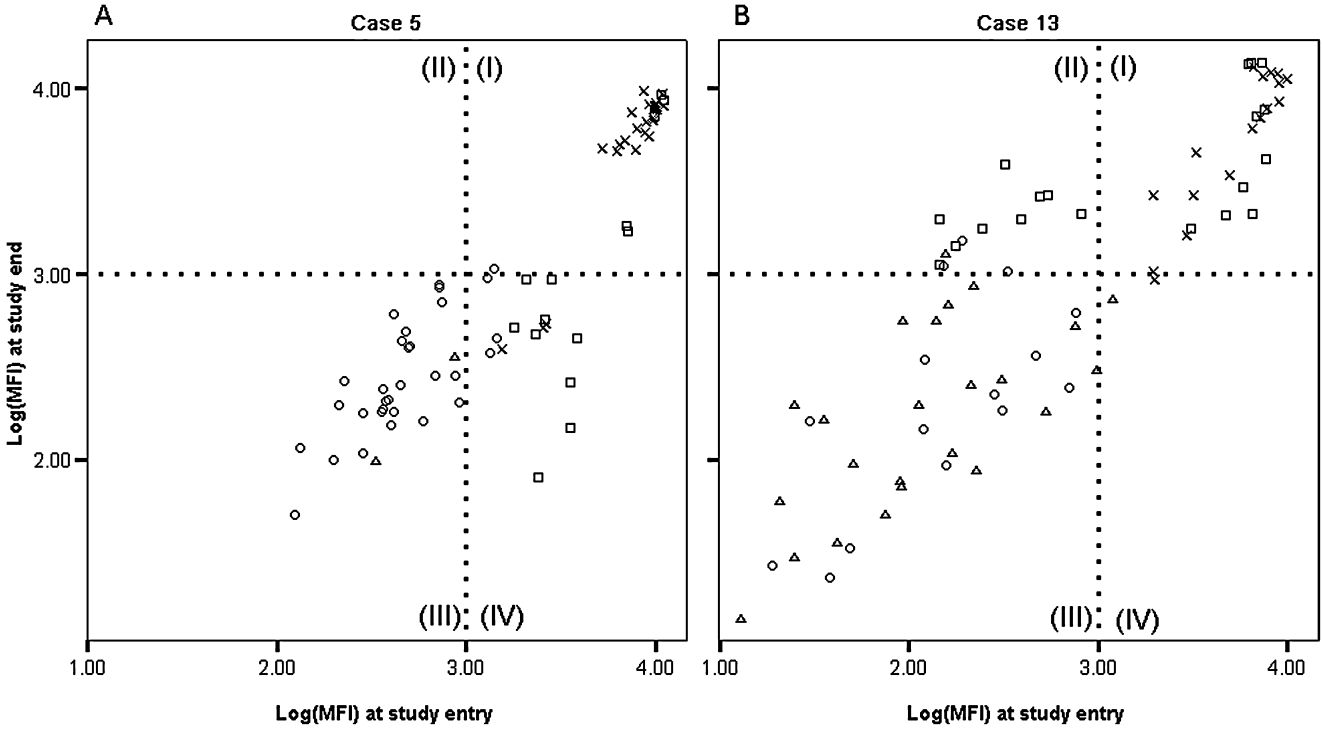

Fig. 3. Comparison of HLA antibody strength at study entry and at study end in Case 5 (A) and Case 13 (B) is shown using scatter plot. MFI was the normalized value of reactive HLA antibody by negative control serum before log transformation. Each data point shows a specific antibody against antigen with different matching category: both EBM and CREG compatible (○), only EBM compatible (△), only CREG compatible (□), and both EBM and CREG incompatible (×). The vertical and horizontal dashed lines correspond to the cutoff values for an antibody-positive reaction. The HLA-specific antigens falling in the third quadrant area (III) of each graph are safe for patients.

was A/BU matched, EBM matched, and finally CREG matched.

The change pattern of HLA specificity antibodies

To determine whether patients develop specific HLA antibodies to EBM-matched antigens after PLT transfusions, we determined HLA antibody specificity of nine patients at study entry and end points. All but two patients showed similar patterns of HLA antibodies at both entry and end points of this study (Fig. S1, available as supporting information in the online version of this paper). Most HLA antibodies existed consistently, except for some HLA antibodies that decreased or disappeared over time. Figure 3 shows the change pattern of reactive antibodies for two cases (Cases 5 and 13). Some HLA antibodies against CREG-compatible antigens decreased in the patient with acute myeloid leukemia (AML; Case 5). The patient with myelodysplastic syndrome (MDS; Case 13) acquired more HLA antibodies that were not detected at study entry as a result of many other blood components administered during the study period. Interestingly, these emerging HLA antibodies were members of HLA-B7 CREG and shared the same public epitope. During this cohort study, no HLA antibodies to the EBM-matched antigens emerged in any of the patients, demonstrating that the use of EBM-matched PLT transfusion is good for HLA-alloimmunized patients.

DISCUSSION

In this study, we prospectively evaluated 142 PLT transfusion outcomes with different matching strategies (A/BU, CREG, and EBM method) in nine HLA-alloimmunized patients and monitored the change of HLA antibody patterns in patients in a time-series follow-up study. The results indicated that the selection of PLTs for HLA-alloimmunized refractory patients by EBM method is as effective as that by A/BU matching, and follow-up antibody analysis confirmed that no specific antibodies were detected against EBM-matched antigens or epitopes in HLA alloimmunization-related refractory patients.

Structural EBM method is a new strategy to assess HLA compatibility of donor and recipient pairings at the amino acid level using HLAMatchmaker software.[6] It considers structurally defined epitopes (triplets or eplets) recognized by antibodies and simultaneously considers public and private epitopes between different HLA-specific antigens. The basic assumption of this algorithm is that alloimmunized patients do not produce antibodies against polymorphic amino acid epitopes on mismatched HLA antigens, if these epitopes are also presented with the patient's own HLA antigens. Nambiar and coworkers[7] have verified this algorithm (triplet version) to determine PLT compatibility in 16 alloimmunized patients with aplastic anemia refractory to random-donor PLT transfusions and found that a threshold of at least nine triplet mismatches appeared to be associated with successful transfusions. Brooks and colleagues[8] have also verified another algorithm (eplets version) to determine PLT compatibility in 73 alloimmunized patients and found eplets mismatches not more than 11 had good PLT transfusion outcomes. However, these studies only used the epitope mismatch number of HLA type to determine the compatibility of donor and recipient. They did not consider if critical epitopes recognized by the patient's antibodies were among these mismatch epitopes. There has been no prospective follow-up study to demonstrate the lack of alloreactivity to these mismatched antigens on subsequent HLA antibody identification test. We first used HLAMatchmaker and single-antigen HLA antibody detection beads to identify acceptable mismatches in HLA-alloimmunized thrombocytopenic patients and selected PLTs that did not contain these critical epitopes for providing effective PLTs.

Previous studies have suggested that PLTs for refractory alloimmunized patients might be selected according to an "antigens or epitopes avoidance" matching strategy based on the patient's HLA antibody results by conventional antibody screening techniques using lymphocyte or purified HLA antigen panels.[5,11] Laundy and colleagues[5] identified HLA-specific antibodies of 13 patients with acquired aplastic anemia and found that the majority of HLA-specific antibodies were against HLA-A antigens. However, conventional antibody screening techniques do not enable widespread analysis of patient sera because each target carries up to six different HLA Class I antigens, creating complex antibody reaction patterns caused by serologic cross-reactivity between HLA-specific antigens. Single-antigen HLA antibody detection beads comprise the full repertoire of serologically defined HLA-A and HLA-B specificities bound to a solid phase that enables accurate detection and characterization of HLA-specific antibodies in patient sera, without the ambiguities encountered using conventional techniques. We used this technique to identify HLA-specific antibodies of 23 patient sera and found that most HLA-specific antibodies were against HLA-B rather than HLA-A antigens in our study population. This discrepancy may be owing to the limitation of the antibody detection system or the size of the study population.

In the results of single-antigen HLA antibody detection beads and epitope prediction analysis by HLAMatchmaker, we usually found three to six critical reactive epitopes of HLA antigens. Most HLA-alloimmunized patients have antibodies whose specificity is restricted to a small number of HLA epitopes that is shared by several HLA specificities. The highly reactive epitopes were 145QRT, 65QIA, 62QE, 127K, and 163EW in multiply transfused patients. These epitopes are well-known public epitopes belonging to 1C, 2C, 5C, 7C, and 10C. The frequencies of these public epitopes were also high (44%-59%) in our PLT donor files. This result was similar to the finding of previous studies in multiply transfused patients with acquired aplastic anemia[5] and in oncology patients.[12] Only one private epitope 151AHA, belonging to HLA-A11 specificities, was found frequently. 151AHA was defined as a strong alloreactive epitope of HLA antigens by Duquesnoy (unpublished data). Because the antigen frequency of A11 was very high (31%) in our donor file, we avoided A11 PLTs for non–A11-type patients. Moreover, 65% of the patients developed intra-CREG antibodies in our study. This is one explanation of why CREG-matched PLTs were not as effective as other matching methods. In addition, 53% of HLA-alloimmunized patients had developed HLA-C antibodies. HLA-C matching was not considered in PLT transfusion practice, as HLA-C antigens are expressed at very low levels on PLTs.[13] The transfusion success rates in this series were still high (approx. 80%) in our study. Therefore, HLA-C antigen matching seems to be unnecessary in choosing compatible PLTs for alloimmunized patients. Besides, we only used low-resolution HLA typing data to pair donor and recipient. High-resolution HLA typing seems to be unnecessary for PLT transfusions.

Although using leukoreduced PLTs has been reported to reduce the prevalence of HLA alloimmunization in the TRAP study,[14] HLA alloimmunization–related refractoriness is still a significant problem in clinical practice. In our study, many patients had leukoreduced PLTs; however,

there were still 31.5% (23/73) of patients who had developed HLA antibodies during PLT transfusion regimen. This incidence rate of HLA alloimmunization was similar to the data reported in a research conducted in Canada before using prestorage leukoreduction blood components.[15] One explanation for our result may be that many patients received nonprestorage leukoreduction RBC components in our hospital. For this high incidence rate of HLA alloimmunization, using only A/BU-matched PLTs could not provide enough PLTs for these patients. Using the EBM strategy could expand the number of compatible PLTs and help in improving the HLA-matched PLT distribution, and universal use of leukoreduction blood components should be further considered for clinical practice.

The patients who were HLA Class I homozygous were HLA alloimmunized in a higher proportion than those who were heterozygous. Individuals who are HLA homozygous might be expected to be more easily sensitized through exposure to nonself HLA antigens. Therefore, HLA homozygous patients to a great extent tend to be highly alloimmunized patients, making it more difficult to select HLA-matched PLTs. In contrast, more HLA-mismatched antigens could be acceptable for HLA heterozygous patients. Most PLT transfusion guidelines have suggested using ABO-matched PLTs for PLT refractory patients when HLA-matched PLTs are not available.[16] However, the importance of ABO-identical or -compatible PLTs is still controversial in clinical practice. In our study, we further determined the effectiveness of 221 HLA-matched PLT transfusions with different ABO matching grades. We were not able to find a significant effect of ABO incompatibility on PLT transfusion success rates of HLA-matched transfusions (data not shown). This emphasizes the importance of the effort to find HLA-matched PLTs for HLA-alloimmunized patients and that it is more important than selecting ABO-identical or -compatible PLTs.

Although the EBM strategy was useful for HLA-alloimmunized patients, there were some limitations that should be considered. First, we used the Luminex HLA single-antigen antibody assay to detect HLA antibodies. This analysis system is a very powerful tool for HLA antibody identification. However, one may question whether the very-low-level antibodies detected by this system are clinically important or not. Further study is needed to clarify the clinical significance of the very-low-level antibodies. Second, epitope prediction is difficult to determine by HLAMatchmaker software, as there are no suitable epitope templates for some individuals. The epitope templates or "patches" of this program need to be finely adjusted for such cases. One particular study identified the HLA exact epitopes on the basis of antibody adsorbing and eluting experiments.[17] We incorporated their reported epitopes and modified the patches of HLA-Matchmaker to solve the epitope identification problems in some of our cases.

In conclusion, the EBM strategy could be used in choosing proper PLTs for refractory patients and no further specific antibodies appeared in these patients after they received mismatched PLTs by this matching strategy.

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CONFLICT OF INTEREST

All authors have no relevant conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Comparison of HLA antibody strength at study entry and at study end in 9 HLA-alloimmunized patients shown using scatter plot. Median fluorescent intensity (MFI) was the normalized value of reactive HLA. Sixty-five different HLA-A (○) and HLA-B (△) specificities were detected. The vertical and horizontal dashed lines correspond to the cutoff values for antibody positive reaction. HLA-specific antigens falling in the third quadrant area (III) of each graph are considered safe for patients.

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