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Research article

Establishment and clinical application of the HLA genotype database of platelet-apheresis donors in Suzhou

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ABSTRACT

The establishment of a platelet-apheresis donor database may provide a feasible solution to improve the efficacy of platelet transfusion in patients with immune platelet transfusion refractoriness (PTR). This study aimed to establish HLA genotype database in Suzhou, to provide HLA-I compatible platelets for PTR patients to ensure the safety and effectiveness of platelet transfusions. We used a polymerase chain reaction sequence-based typing (PCR-SBT) method to establish the database by performing high-resolution HLA-A, -B, and –C genotyping on 900 platelet-apheresis donors. HLA-I antibody was detected in patients using a Luminex device, and HLA-I gene matching was performed by an HLA-Matchmaker. We found that the highest frequency of the HLA-A allele was A*11:01 (17.06 %), followed by A*24:02 (14.67 %) and A*02:01 (13.61 %). The highest frequency of the HLA-B allele was B*46:01 (9.78 %), followed by B*40:01 (8.39 %) and B*13:02 (33 %). After the detection of platelet antibodies in 74 patients with immune PTR, we found 30 HLA-A antibodies and 48 HLA-B antibodies, and there were a variety of high frequency antibodies whose alleles were low in the donor database, such as HLA-A*68:02, and B*57:01. After avoiding donor-specific antibodies (DSA) matching, 102 of 209 platelet-compatible transfusions were effective, resulting in an effective rate of 48.8 %, which significantly improved the efficacy of platelet transfusion. The establishment of a platelet donor database is of great significance to improve the therapeutic effect of platelet transfusion in patients with hematologic disorder, and save blood resources, and it is also the premise and guarantee of precise platelet transfusion.

1. Introduction

Platelet transfusion refractoriness (PTR), a challenging issue in clinical platelet transfusion, is caused by nonimmune and immune factors. Nonimmune PTR is more common (80–90 % of cases), and occurs primarily through fever, infection, splenomegaly, bleeding,

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drugs, and other factors. Immune PTR is related to HLA-I, HPA, CD36, and other factors, thus being less common (20–25 % of cases). Notably, HLA-I antibody is the most important factor leading to immune PTR (about 80–90 % of cases) [1–3]. Due to the strong immunogenicity and high polymorphism of HLA-I antigen, the positive rate of HLA-I antibody easily increases in patients with multiple platelet transfusions, which increases the risk of PTR, and increases the complexity of platelet HLA-I gene matching [4]. At present, the main clinical strategies for PTR caused by HLA-I antibodies include HLA-I gene matching in addition to platelet serological cross-matching [2,5,6]. HLA-I gene matching can prevent and improve immune PTR and thus provide a precise platelet transfusion scheme for clinical practice. However, it is necessary to establish a large database of HLA-I platelet donors to provide genotype-compatible platelets in a timely manner, thereby improving clinical platelet transfusion efficacy [7,8]. Based on the development of platelet genotypes in China and abroad, to improve the effectiveness of platelet transfusion for hematological disease patients in Suzhou, the Suzhou Blood Center started the construction of a platelet HLA-I donor database in 2021. A database of 900 known HLA-A, -B, and –C genotypes has been established, and corresponding HLA-I gene matching was carried out in clinical practice to improve the efficacy of platelet transfusion, to promote further development of precise platelet transfusion.

2. Material and methods

2.1. Sample source

A total of 900 platelet-apheresis donors who donated more than three times a year for three consecutive years in the Suzhou Blood Center during 2021–2023 were selected. The donors were aged 18–50 years old, and they included 867 males and 33 females. There were 200 donors with type A, 174 donors with type B, 425 donors with type O, and 101 donors with type AB. Anticoagulant whole-blood samples were collected for genotyping at the HLA-A, -B, and –C.

In addition, platelet HLA-I genotyping was performed on 74 samples of PTR patients sent to our institution from the First Affiliated Hospital of Soochow University from March 22, 2022, to August 21, 2023. These patients included 28 males and 46 females with diagnoses of acute myeloid leukemia (44 cases), acute lymphoblastic leukemia (6 cases), myelodysplastic syndrome (13 cases), lymphoma (5 cases), hemophagocytic syndrome (2 cases), aplastic anemia (3 cases), and pancytopenia (1 case).

2.2. DNA extraction

DNA extraction and purification of 200 μL anticoagulant whole-blood samples were performed in accordance with the instructions of TGuide S32 Magnetic Blood DNA kit 3 (No. KJLTY1313; TianGen Biotech (Beijing) Co., Ltd., China).

2.3. HLA-I genotyping

HLA-I genotyping was performed by PCR-SBT in accordance with the instructions of the genotyping test kit (No. 202207A; Jiangsu Weihe Biotechnology Inc., China). In simple terms, the extracted DNA molecules were amplified by PCR (ABI 9700; Applied Biosystems, USA), and then the PCR products were purified and sequenced after electrophoresis confirmation. The HLA-I gene high-resolution typing information was obtained by AccuType SBT Analysis Software. The basic amplification PCR reaction parameters were as follows: primary denaturation 95 °C/5 min, followed by 36 cycles for 93 °C/30 s, 63 °C/40 s, 72 °C/150 s, and final extension 72 °C/5 min. The sequencing PCR reaction parameters were as follows: primary denaturation 96 °C/1 min, followed by 25 cycles for 98 °C/25 s, 60 °C/150 s. The effect of HLA-C alleles on HLA-I matching has not yet been determined, so HLA-C allele data are not presented in the results section.

3. Platelet antibody detection with Luminex

HLA-I antibodies were detected in the patient's plasma in accordance with the instructions of the LIFFCODES LSA™ CLASS I kit (No. 3012154; Immucor; Beijing Bofurui Gene Diagnostic Technology Co., Ltd., China), and fluorescence capture of the specific binding beads by the Luminex 200 multifunction flow cytometer (FLEXMAP-3D, ThermoFisher Scientific Inc., USA). The antibody result was judged according to the mean fluorescence intensity (MFI) of the beads, where MFI $<$500 was considered negative, while MFI $\geq$500 was regarded as positive.

3.1. Platelet HLA-I gene matching

According to the ABO isotype and donor-specific antibodies (DSA) avoidance principle, allele matching of clinical patients was performed using the established 900 cases of the HLA-I donor database. Compatible donor platelets were selected based on the HLA-epitope strategy for coordinated transfusion. HLA-epitope mismatch strategy was executed using HLA-Matchmaker software, which defined epitopes in a 'search engine', to facilitate the process of HLA antigen and/or epitope–based platelet selection in platelet transfusion. The software performed compatibility tests by matching the antigen and epitope of the HLA-I locus of the donor and the patient. Namely, based on the uploaded HLA high-resolution gene sequencing results of both the donor and the patient (e.g., HLA-A*11:01, A*24:02; HLA-B*41:06, B*15:01; HLA-C*04:07, C*02:02), and the antibody information in the patient were automatically matched, and the most compatible donor platelets were selected for coordination transfusion according to the principle of minimized epitope mismatch and maximized gene coincidence [9,10].

3.2. Evaluation efficiency of platelet HLA-I gene matching

The corrected count increment (CCI) is generally used to evaluate PTR. It is a formula that includes the difference in platelet count after transfusion, body surface area, and platelet dose. As shown below, CCI = (post-transfusion platelet count − pre-transfusion platelet count) × 10^{11} × BSA (m^2)/platelets transfused (10^{11}). BSA was determined as follows: BSA (m^2) = 0.0061 × height (cm) + 0.0128 × weight (kg) − 0.01529. CCI can be measured at any time after transfusion. As reported in the literature, 14-h CCI is also used to diagnose PTR in our hospital. Usually the platelet transfusion is completed at 5−6 p.m. and the sample is collected at approximately 8 a.m. the next morning for platelet counting, that is, the CCI value is calculated 14 h after the platelet transfusion [11]. The PTR criterion is 14-h CCIs below 5 on two consecutive occasions.

3.3. Statistical analysis

Statistical analysis was performed in GraphPad Prism 8.0. Student's *t*-test was used to compare platelet counts before and after transfusion, and $P < 0.05$ was considered statistically significant. The measurement data were presented as mean ± standard deviation (SD).

4. Results

4.1. HLA-A, and -B genotyping and frequency

The frequencies of HLA-A, and -B alleles are shown in Table 1 and 2. There were 38 alleles in the HLA-A locus in the donor database, and the highest frequencies were those of A*11:01 (17.06 %), A*24:02 (14.67 %), and A*02:01 (13.61 %). There were 73 alleles in the HLA-B locus, with the highest frequencies being those of B*46:01 (9.78 %), B*40:01 (8.39 %), and B*13:02 (7.33 %) (see).

4.2. Serological frequency distribution of HLA-A, and -B antigens

The serological frequency distribution of HLA-A and -B antigens is shown in Table 3. There were 19 types of HLA-A antigen phenotypes, and the highest frequency of HLA-A antigen was that of HLA-A2 (27.94 %), followed by HLA-A11 (18.78 %) and A24 (15.11 %). In addition, the phenotype frequency of the HLA-A203 antigen in the database was high, accounting for 2.72 %. There were 39 types of HLA-B antigen phenotypes, and the highest frequency of HLA-B antigen was that of HLA-B13 (11.67 %), followed by HLA-B46 (9.83 %) and B60 (8.39 %) .

4.3. Platelet HLA-I antibody detection in patients

Platelet antibodies directly affect the effectiveness of platelet transfusion. Patients with PTR should be screened for platelet antibodies after excluding nonimmune factors, and then allelic matching with HLA-epitope should be performed using HLA-Matchmaker based on avoiding DSA. Specific and diverse HLA-A and B antibodies were detected in 48 of 74 multiple PTR patients, with a total of 30 HLA-A allele antibodies and 48 HLA-B allele antibodies detected (Table 4). Antibodies of the HLA-A*68:02 (43.75 %) and B*57:01 (47.92 %) were the most common, but the frequencies of these two alleles in the donor database were 0.17 % and 1.78 %, respectively. In contrast, HLA-11:01 had the highest proportion in the donor database (17.06 %), but antibody to this allele was detected in 16.67 %

Table 1
Allele frequency distribution of HLA-A in platelet donors.

HLA-A	n	Frequency (%)	HLA-A	n	Frequency (%)
A*11:01	307	17.06	A*24:03	4	0.22
A*24:02	264	14.67	A*03:02	4	0.22
A*02:01	245	13.61	A*69:01	4	0.22
A*33:03	171	9.50	A*68:02	3	0.17
A*02:07	148	8.22	A*01:03	2	0.11
A*30:01	136	7.56	A*24:07	2	0.11
A*02:06	103	5.72	A*30:04	2	0.11
A*31:01	82	4.56	A*30:02	2	0.11
A*01:01	71	3.94	A*02:02	1	0.06
A*02:03	49	2.72	A*02:09	1	0.06
A*03:01	49	2.72	A*02:53 N	1	0.06
A*26:01	41	2.28	A*11:56	1	0.06
A*11:02	31	1.72	A*24:04	1	0.06
A*32:01	21	1.17	A*25:01	1	0.06
A*29:01	18	1.00	A*26:03	1	0.06
A*68:01	14	0.78	A*29:02	1	0.06
A*02:10	6	0.33	A*33:01	1	0.06
A*02:05	5	0.28	A*34:01	1	0.06
A*24:20	5	0.28	A*66:01	1	0.06

Table 2
Allele frequency distribution of HLA-B in platelet donors.

HLA-B	n	Frequency (%)	HLA-B	n	Frequency (%)	HLA-B	n	Frequency (%)
B*46:01	176	9.78	B*15:18	20	1.11	B*35:05	2	0.11
B*40:01	151	8.39	B*67:01	18	1.00	B*15:19	2	0.11
B*13:02	132	7.33	B*44:02	16	0.89	B*15:12	2	0.11
B*58:01	119	6.61	B*35:03	15	0.83	B*15:05	2	0.11
B*15:01	113	6.28	B*07:05	15	0.83	B*14:01	2	0.11
B*51:01	97	5.39	B*08:01	15	0.83	B*48:03	2	0.11
B*13:01	78	4.33	B*15:27	12	0.67	B*15:03	1	0.06
B*44:03	58	3.22	B*50:01	12	0.67	B*15:08	1	0.06
B*54:01	57	3.17	B*38:01	9	0.50	B*15:13	1	0.06
B*35:01	55	3.06	B*56:01	9	0.50	B*15:21	1	0.06
B*40:06	54	3.00	B*18:01	6	0.33	B*15:25	1	0.06
B*38:02	53	2.94	B*35:02	6	0.33	B*15:58	1	0.06
B*48:01	49	2.72	B*14:02	5	0.28	B*18:02	1	0.06
B*52:01	45	2.50	B*39:05	5	0.28	B*27:06	1	0.06
B*55:02	41	2.28	B*27:07	4	0.22	B*27:10	1	0.06
B*15:02	38	2.11	B*15:07	4	0.22	B*39:24	1	0.06
B*40:02	35	1.94	B*15:32	7	0.39	B*46:09	1	0.06
B*15:11	33	1.83	B*81:02	4	0.22	B*53:01	1	0.06
B*57:01	32	1.78	B*15:17	3	0.17	B*55:01	1	0.06
B*37:01	32	1.78	B*35:08	3	0.17	B*55:04	1	0.06
B*07:02	30	1.67	B*40:03	3	0.17	B*55:12	1	0.06
B*39:01	29	1.61	B*47:01	3	0.17	B*56:10	1	0.06
B*51:02	24	1.33	B*51:08	3	0.17	B*59:01	1	0.06
B*27:05	20	1.11	B*49:01	2	0.11	B*81:01	1	0.06
B*27:04	20	1.11						

of the patients. This also fully indicates that DSA should be preferentially avoided during platelet matching, and platelets with the most compatible alleles and fewer mismatches should be selected for transfusion.

4.4. Analysis of eplet specificity of DSA

We analyzed the eplets that were HLA-I antibody-verified on the website-based HLA Epitope Registry (http://www.epregistry.com.br/), and reviewed the literature on high-immunogenicity eplet statistics [12]. The eplets of the donor database with allele frequency below 1 %, but HLA-A antibody frequency above 25 % and HLA-B antibody frequency above 35 % (22 in total), were compared with the eplets of the low-immunogenicity HLA-A*11:01 (Table 5). We found that most of high antibody frequency HLA molecules contained one or more eplets with high-immunogenicity scores (*sc.* $>$ 0.2; 41T, 45 KE, 62LQ, 80I, 82LR, 127K, 131S, 144TKH, 145KHA, marked in red), but with the exception of HLA-B*08:01 and HLA-B*81:01, the frequency of antibody production is 41.67 %.

4.5. Clinical application of the platelet HLA-I donor database

The establishment of the HLA-I donor database aimed to match HLA-I genotypes in patients with multiple PTR and improve the effectiveness of platelet transfusion. Since the establishment of the database, platelet HLA-I gene matching has been performed 293 times on 74 clinical patients after avoiding DSA. Of these, the matching was done for actual clinical use 209 times. The effective rate of platelet transfusion was 48.8 % (Table 6 and 7), which significantly increased the platelet count after transfusion ($P < 0.0001$) and improved the clinical platelet transfusion efficacy in the patients with immune PTR.

4.6. Clinical retrospective analysis of special cases

After the transfusion of clinically matched platelets, we detected HLA-A2 alloimmune antibody in one patient (HLA-A*02:03, A*31:01; B*15:25, B*27:05; C*04:03, C*02:02). The patient had a variety of HLA antibodies before HLA-I matching, but no HLA-A2 antibody. Of the six HLA-I matches after DSA evading, four were HLA-A2 serologic donor platelets (Table 8). HLA-A2 antibody was detected 20 days after platelet transfusion. Comparative analysis of the patient and donor HLA eplets (HLA-A*02:03 vs HLA-A*02:01, A*02:06, and A*02:07) showed that HLA-A*02:03 was the same as other eplets of HLA-A*02:01, A*02:06, and A*02:07, but different eplets 149TAH (HLA-A*02:03) and 145KHA (HLA-A*02:01, A*02:06, and A*02:07) were generated at the 149th position due to different amino acids. 145KHA is a high-immunogenicity eplet, so the patient produced specific HLA-A2 antibody after multiple transfusions of serologically homogeneous platelets with HLA-A2 phenotype. We also believe that structural differences due to different amino acids will eventually manifest as different eplets, which will lead to epitope mismatch.

5. Discussion

The Suzhou Blood Center has established a database of 900 platelet-apheresis donors, including HLA-I, HPA, and CD36 data (HPA,

Table 3
Serological distribution of HLA-A and -B antigens in platelet donors.

HLA-A	n	Frequency (%)	HLA-B	n	Frequency (%)
A2	503	27.94	B13	210	11.67
A11	338	18.78	B46	177	9.83
A24	272	15.11	B60	151	8.39
A33	172	9.56	B62	140	7.78
A30	140	7.78	B58	119	6.61
A31	82	4.56	B51	100	5.56
A1	73	4.06	B61	92	5.11
A3	53	2.94	B35	81	4.50
A203	49	2.72	B44	74	4.11
A26	42	2.33	B75	73	4.06
A32	21	1.17	B38	62	3.44
A29	19	1.06	B54	57	3.17
A68	17	0.94	B48	51	2.83
A210	6	0.33	B27	46	2.56
A2403	4	0.22	B52	45	2.50
A69	4	0.22	B55	45	2.50
A34	1	0.06	B7	45	2.50
A66	1	0.06	B37	32	1.78
A25	1	0.06	B57	32	1.78
			B3901	29	1.61
			B5102	24	1.33
			B71	20	1.11
			B67	18	1.00
			B8	15	0.83
			B50	12	0.67
			B56	9	0.50
			B18	7	0.39
			B39	6	0.33
			B65	5	0.28
			B81	5	0.28
			B76	4	0.22
			B47	3	0.17
			B63	3	0.17
			B49	2	0.11
			B64	2	0.11
			B72	1	0.06
			B77	1	0.06
			B53	1	0.06
			B59	1	0.06
Total	1798	99.89		1800	100

Note: A*11:56/A*02:53 N no serotype.

and CD36 data are not shown), and preliminary platelet coordination transfusion has been carried out with the combination model of avoiding DSA and HLA-epitope. Since ABO isotypic random platelet transfusion is the routine clinical strategy, and the patients also have some non-immune factors such as fever and infection, a large proportion of patients experience the PTR phenomenon. Serological cross-matching or HLA-I genotype platelet transfusion is used only after the occurrence of PTR [13]. Therefore, the patients we performed HLA-I gene matching were patients with multiple PTR, who may have produced a complex of platelet antibodies. The effectiveness of platelet transfusion is measured by the CCI value, so using our combined matching strategy, the effective rate of platelet transfusion in the patients with multiple PTR was increased by 48.8 %, where the highest rate of 14-h CCI was 40.5. The platelet count increased and bleeding improved, showing a good clinical transfusion effect, which not only ensured the safety and effectiveness of blood transfusion but also reduced the waste of platelet resources.

In our platelet database, the highest allele frequency of HLA-A was that of A*11:01 (17.06 %), which was slightly lower than the common proportion of the Chinese population (21.21 %). Allele frequencies greater than 1 % included those of A*24:02, A*02:01, A*33:03, A*02:07, A*30:01, A*02:06, A*31:01, A*01:01, A*02:03, A*03:01, A*26:01, A*11:02, A*32:01, and A*29:01. The highest allele frequency of HLA-B was that of B*46:01 (9.78 %). There were 26 allele frequencies greater than 1 %, among which those of B*40:01, B*13:02, B*58:01, B*15:01, and B*51:01 were the highest. Through the comparative study, we found that the frequency distribution of HLA-A in the database was close to that of the Common alleles and Well-documented alleles (CWD table, version 2.2) in the Chinese population, while the frequency of HLA-B alleles was slightly different from the CWD table. For example, HLA-B*15:01 and HLA-B*15:02 are relatively common HLA-B alleles in the Chinese population, accounting for 4.73 and 3.63 % of cases, respectively. In the Suzhou platelet database, the frequency of HLA-B*15:01 was high (6.28 %), while the proportion of HLA-B*15:02 was relatively low (2.11 %). The HLA-A frequency distribution is also close to that previously reported by Deyi Xu et al. [3], while the frequency distribution of HLA-B alleles is slightly different from the reported one, indicating that the more extensive polymorphism of HLA-B genes could lead to the differences in the frequencies of some common alleles in different provinces and cities. Therefore, provinces and cities need to establish regional donor databases to lay the foundation for personalized diagnosis and treatment programs and

Table 4
Frequency distribution of HLA antibodies in patients.

HLA-A	Frequency (%)	HLA-B	Frequency (%)	HLA-B	Frequency (%)
A*68:02	43.75	B*57:01	47.92	B*27:03	31.25
A*24:02	37.50	B*58:01	47.92	B*39:01	31.25
A*23:01	35.42	B*08:01	41.67	B*40:02	31.25
A*24:03	35.42	B*15:12	41.67	B*41:01	31.25
A*68:01	35.42	B*54:01	41.67	B*44:02	31.25
A*25:01	33.33	B*81:01	41.67	B*55:01	31.25
A*66:02	33.33	B*07:02	39.58	B*73:01	31.25
A*34:02	31.25	B*15:16	39.58	B*15:02	29.17
A*32:01	29.17	B*49:01	39.58	B*15:03	29.17
A*43:01	27.08	B*56:01	39.58	B*37:01	29.17
A*03:01	25.00	B*07:03	37.50	B*42:01	29.17
A*33:03	25.00	B*27:08	37.50	B*47:01	27.08
A*02:02	22.92	B*35:01	37.50	B*78:01	27.08
A*02:05	22.92	B*35:08	37.50	B*15:01	22.92
A*66:01	22.92	B*38:01	37.50	B*48:01	22.92
A*69:01	22.92	B*40:01	37.50	B*14:01	20.83
A*02:01	20.83	B*44:03	37.50	B*14:02	14.58
A*02:03	20.83	B*53:01	37.50	B*46:01	8.33
A*11:02	20.83	B*59:01	37.50		
A*26:01	18.75	B*82:02	37.50		
A*31:01	18.75	B*50:01	35.42		
A*80:01	18.75	B*27:05	33.33		
A*01:01	16.67	B*45:01	33.33		
A*11:01	16.67	B*51:01	33.33		
A*33:01	16.67	B*52:01	33.33		
A*74:01	16.67	B*67:01	33.33		
A*29:01	14.58	B*13:02	31.25		
A*29:02	14.58	B*15:13	31.25		
A*30:01	10.42	B*15:18	31.25		
A*36:01	10.42	B*18:01	31.25		

promote the further development of transfusion medicine in society. Only by knowing the characteristics of HLA genotyping in the local population can we better serve clinical patients. In addition, it is appropriate to expand the donor database in combination with the Chinese hematopoietic stem cell donor database to facilitate the recruitment of donors with rare HLA genotypes and alleviate the problem of regional allelic differences.

The highest serological frequency of HLA-A antigen was that of HLA-A2 (27.94 %), and the key antigen of HLA-A2 subtype HLA-A203 had a high proportion (2.72 %). In addition, the patients with HLA-A203 in clinical platelet gene matching produced HLA-A2 antibody due to transfusion of HLA-A2 platelets. The production of HLA-A2 antibodies was mainly due to the mismatch of eplets. Eplets are functional units of the donor HLA epitopes, which are key amino acids located on the surface of HLA molecules to determine antigen polymorphism. The epitopes defined by eplets play an important role in gene mismatch, which can be immune targets of DSA in the context of transplantation or blood transfusion [6,12,14]. Previous studies have shown that because of the strong immunogenicity, 145KHA eplet is a high-frequency eplet that easily produces antibodies as well as an unacceptable mismatch eplet for HLA-A*02:03 [12,15]. HLA-A2 allelic differences have been reported to have an important impact on acute graft-versus-host disease and survival in unrelated hematopoietic stem cell transplantation, with a significantly increased risk of death after transplantation in non-compatible HLA-A*02:01/HLA-A*02:06 donors [16]. According to a growing number of studies, HLA-A*02:01 with HLA-A*02:03 and HLA-A*02:06 are unacceptable HLA mismatch, so it is necessary to be vigilant about the mismatch of HLA-A2 subtypes in both hematopoietic stem cell transplantation and platelet matching applications [17,18]. Therefore, it is necessary to conduct high-resolution genotyping, and when performing platelet HLA-I matching, epitope matching using HLA-Matchmaker while avoiding DSA can maximize the selection of genetically compatible platelets and thus improve the efficacy of platelet transfusion [9,19]. As Duquesnoy RJ et al. [15] showed that determining HLA compatibility at the allelic level is a more accurate method to identify suitable donors for highly sensitized patients. This can increase the success rate of transplantation and improve the long-term survival rate of grafts. In addition, Mayor NP et al. [20] pointed out that matching exons, introns, and non-translated regions outside antigen-recognition sites with high-resolution genotyping can significantly improve the prognosis of hematopoietic stem cell transplantation patients; thus, high-resolution genotyping should be prospectively performed at donor selection.

Eplets are closely related to the degree of antibody formation. The immunogenicity of different eplets is very different, so not every eplet mismatch can induce antibody production. The relationship between the number of mismatched eplets and antibodies is as follows: if there are more mismatched eplets, recipients are more likely to be exposed to high immunogenic eplets, and the possibility of antibody generation is greater [21]. A highly immunogenic eplet mismatch was sufficient to induce DSA production, as evidenced by the production of HLA-A2 allele antibodies. *Kram*er CSM et al. [4] pointed out that the probability of HLA allele mismatch inducing antibodies depends on the frequency of occurrence of immunogenic eplets in the population. If this frequency is higher, the donor and recipient are more likely to share the epitope, and less likely to have a corresponding specific antibody. If highly immunogenic eplets occur less frequently in the population, there is a higher likelihood of developing specific antibodies against this epitope in the case of

Table 5
Analysis of HLA immunogenic eplets.

Alleles	Antibodyfrequency(%)	Donordatabasefrequency(%)	Eplets																
A*11:01	***16.67***	***17.06***	***62QE***	**65RNA**				**79 GT**		***90D***	***138MI***	**144 KR**	***144K***		***149AH***	***150AAH***	***163R***	**163RW**		***150AHA***
A*68:02	**43.75**	**0.17**	**62RR**	**65RNA**				**79 GT**		**127K**		**144TKH**	**144K**	**145KHA**	**149AH**	**150AAH**			**193AV**	**253Q**
A*23:01	**35.42**	**none**	**62 EE**	**65 GK**				**80I**	**82LR**	**127K**	**138MI**							**166DG**		
A*24:03	**35.42**	**0.22**	**62 EE**	**65 GK**				**80I**	**82LR**	**127K**	**138MI**	**144 KR**	**144K**		**149AH**	**150AAH**				
A*68:01	**35.42**	**0.78**	**62RR**	**65RNA**						**127K**		**144TKH**	**144K**	**145KHA**	**149AH**	**150AAH**			**193AV**	**253Q**
A*25:01	**33.33**	**0.06**	**62RR**	**65RNA**			**76ESI**	**80I**	**82LR**	**90D**	**138MI**			**145RT**	**149TAH**		**163R**	**163RW**	**193AV**	**253Q**
A*66:02	**33.33**	**none**	**62RR**	**65RNA**							**138MI**			**145RT**	**149TAH**			**163 EW**	**193AV**	**253Q**
A*34:02	**31.25**	**none**	**62RR**	**65RNA**						**90D**	**138MI**			**145RT**	**149TAH**				**193AV**	**253Q**
A*43:01	**27.08**	**none**	**62LQ**	**65RNA**			**76ANT**			**90D**	**138MI**			**145RT**	**149TAH**		**163R**	**163RW**	**193AV**	**253Q**
B*08:01	**41.67**	**0.83**			**69TNT**	**71TTS**	**76ESN**	**80N**											**180E**	**156DA**
B*15:12	**41.67**	**0.11**	**44RMA**		**69TNT**	**71TTS**	**76ESN**	**80N**			**131S**							**166DG**		
B*81:01	**41.67**	**0.06**		**65QIA**	**69AA**	**70IAQ**	**76ESN**	**80N**				**143S**						**163 EW**	**180E**	
B*15:16	**39.58**	**none**	**44RMA**	**65RNA**	**69AA**	**70SA**	**62RR**	**80I**	**82LR**		**131S**							**163LW**		
B*49:01	**39.58**	**0.11**	**41T**	**45 KE**	**69TNT**			**80I**	**82LR**		**131S**							**163LW**		
B*56:01	**39.58**	**0.5**		**65QIA**	**69AA**	**70IAQ**	**76ESN**	**80N**			**131S**							**163LW**		
B*07:03	**37.5**	**none**			**69TNT**	**71TTS**	**76ESN**	**80N**										**163 EW**	**180E**	
B*27:08	**37.5**	**none**		**65QIA**	**69AA**		**76ESN**	**80N**			**131S**							**163 EW**		
B*35:08	**37.5**	**0.17**	**44RT**		**69TNT**	**71TTS**	**76ESN**	**80N**			**131S**							**163LW**	**193 PV**	
B*38:01	**37.5**	**0.5**			**69TNT**			**80I**	**82LR**		**131S**									**158T**
B*53:01	**37.5**	**0.06**	**44RT**		**69TNT**			**80I**	**82LR**		**131S**							**163LW**	**193 PV**	
B*59:01	**37.5**	**0.06**			**69TNT**			**80I**	**82LR**		**131S**									
B*82:02	**37.5**	**none**		**65QIA**	**69AA**	**70IAQ**	**76ESN**	**80N**			**131S**							**163LS**		**156DA**
B*50:01	**35.42**	**0.67**	**41T**	**45 KE**	**69TNT**	**71TTS**	**76ESN**	**80N**			**131S**							**163LW**		

Table 6
Platelet HLA-I gene matching in clinical patients.

Patients (N)	Number of HLA-I genotypes	Number of clinical uses	Clinical utilization rate (%)	Number of effective infusion	Effective infusion ratio (%)
74	293	209	71.3	102	48.8

Table 7
Therapeutic effect of platelet HLA-I gene matching infusion.

	Pre-transfusion	Post-transfusion	P value
PLT count ($\times 10^9$/L)	1–32 (11.24 ± 6.61)	1–78 (20.29 ± 13.99)	<0.0001
95 % CI	10.34–12.15	18.38–22.20	
14-h CCI	−7.08–40.50 (6.30 ± 7.78)		

Note: The number is always a range from a minimum to max. CI: confidence interval; CCI: corrected count increment.

Table 8
HLA-I genotypes in patient and donors.

No.	HLA genotype					
Patient	A*02:03	A*31:01	B*15:25	B*27:05	C*04:03	C*02:02
Donor 1	A*02:06	A*31:01	B*35:01	B*51:02	C*04:01	C*15:02
Donor 2	A*02:01	A*02:01	B*15:01	B*35:01	C*03:03	C*03:03
Donor 3	A*02:07	A*29:01	B*07:02	B*46:01	C*01:02	C*07:02
Donor 4	A*02:07	A*31:01	B*15:11	B*44:03	C*03:03	C*07:02

eplet mismatch. Therefore, HLA-epitope matching should be weighted according to the risk degree of each eplet to prevent the high immunogenicity of eplet mismatch and thus improve the approach to the HLA-epitope mismatches for pre-transplant or pre-transfusion risk assessment in patients.

Since the establishment of our database, we have displayed the platelet donation information of the enrolled donors on the same day and conducted platelet gene matching with clinical patients according to their known HLA-I genotype information, which has been different from the previous method of recruiting enrolled blood donors by 'telephone' and collecting platelets. Our approach ensures the timeliness and effectiveness of clinical platelet HLA-I matching transfusion. However, among our platelet matching cases, 42 % of the patients with multiple HLA antibodies were able to find compatible donor platelets through HLA-epitope matching after evading antibodies, whereas 58 % of the patients failed to match due to the inability to evade antibodies. Therefore, a large number of patients could not be matched with compatible donor platelets due to the complexity of antibodies, which reduced the effectiveness of platelet transfusion to a certain extent. At present, we have 900 donor databases, and the small pool capacity also leads to the failure to match compatible platelets for these patients. Therefore, cross-matching negative platelets can only be selected for transfusion outside the donor database through platelet cross-matching. However, cross-matching blood may further lead to the generation of HLA antibodies and increase the difficulty of platelet matching. As such, for patients who need long-term platelet transfusion, it is advisable to advocate prophylactic platelet transfusion; that is, patients who need multiple platelet transfusions according to clinical assessment should use HLA-I compatible platelet transfusion at the first platelet transfusion to prevent alloimmune reactions and reduce the production of HLA-I antibodies, as well as to extend the interval between platelet transfusions.

The establishment of the platelet donor database aimed to provide a feasible method to solve the immune PTR of some patients. Understanding the polymorphism of HLA genes in local blood donors can also predict the risk of platelet transfusion associated with an alloimmune response, which is of great significance for accurate platelet transfusion. However, the establishment of the platelet donor database is labor-intensive and requires the maintenance of a large number of available HLA genotyped apheresis donors, so we will continue to expand the database capacity to fully meet the needs of clinical patients. Of course, this is where we need to improve, because based on the enrollment conditions of our current HLA donor database, it is objectively true that there are more male than female platelet-apheresis donors. In the future, we will also include more female blood donors to balance the issue of gender bias. In addition, we will continue to explore efficient HLA-I gene sequencing methods, to combine next-generation sequencing and nanopore sequencing techniques to conduct high-resolution genotyping of donor and recipient, so as to cope with the ambiguous results produced by PCR-SBT technology. HLA typing at the allelic level can provide more accurate data and a clear vision for HLA genotype matching. Knowledge of epitope specificity could enable a more cost-effective future approach to avoid specific 'epitope' leading to antibody production, improve the effectiveness of clinical platelet transfusion, and further promote the development of transfusion medicine.

Ethics statement

This study was reviewed and approved by the Medical Ethics Committee of Suzhou Blood Center, with the approval number: SZBC202101. Written informed consent was obtained from all participants to participate in the study.

Data availability statement

Not applicable. Data included in article/supp. material/referenced in article.

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CRediT authorship contribution statement

Honghong He: Writing – original draft, Methodology, Investigation, Formal analysis. **Jingjing Huang:** Writing – original draft, Methodology, Investigation, Formal analysis. **Yuanling Zuo:** Visualization, Resources. **Yihan Wang:** Resources, Investigation. **Min Jiang:** Visualization, Resources. **Yiming Jin:** Resources, Investigation. **Longhai Tang:** Writing – review & editing, Supervision, Software, Project administration, Funding acquisition. **Mingyuan Wang:** Writing – review & editing, Supervision, Software, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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