Ensuring HLA-matched platelet support requires an ethnic diverse donor population

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BACKGROUND: Patients refractory for platelet transfusions benefit from human leukocyte antigen (HLA)-matched platelet transfusions. Differences in ethnic background of patients and donors could hamper the availability of sufficient numbers of HLA-matched donors for all patients. We evaluated our HLA-matched donor program and explored the role of ethnic background of patients related to the number of available donors.

METHODS: We performed a cohort study among consecutive patients who received HLA-matched platelet concentrates in the Netherlands between 1994 and 2017. The number of available matched donors was determined per patient. Haplotypes were constructed from genotypes with computer software (PyPop). Based on haplotypes, HaploStats, an algorithm from the National Marrow Donor Program, was used to assess the most likely ethnic background for patients with 5 or fewer and 30 or more donors.

RESULTS: HLA typing was available for 19,478 donors in September 2017. A total of 1206 patients received 12,350 HLA-matched transfusions. A median of 83 (interquartile range, 18-266) donors were available per patient. For 95 (10.3%) patients, 5 or fewer donors were available. These patients were more likely to have an African American background, whereas patients with 30 or more donors were more often from Caucasian origin, compared with Caucasian origin for patients with 30 donors.

CONCLUSION: Adequate transfusion support could be guaranteed for most but not all refractory patients. More non-Caucasian donors are required to ensure the availability of HLA-matched donors for all patients in the Netherlands.

Treatment of hematologic malignancies comprises intensive chemotherapy leading to periods of aplasia, which renders the patient transfusion dependent for prolonged periods of time. A subset of these patients develops refractoriness for random platelet transfusions.

In the Dutch Blood Transfusion Guideline, this is defined as 1-hour corrected count increment (1hCCI) of 7.5 or less after two subsequent ABO-compatible platelet transfusions.[1] The corrected count increment was calculated according to the formula: platelet count increment ($\times 10^9$/L) $\times$ body surface area (m^2)/number of platelets transfused ($\times 10^{11}$).

Refractoriness is associated with an increased risk of bleeding, prolonged hospital stay, and higher hospital costs.[2,3] In 80% to 90% of platelet refractory patients, low 1hCCIs are caused by nonimmunological factors. In 10% of patients, immune-mediated clearance of transfused platelets is the predominant cause of refractoriness. Alloantibodies directed against human leukocyte antigen (HLA) Class IA or

ABBREVIATIONS: 1hCCI = 1-hour corrected count increment; HWE = Hardy-Weinberg equilibrium.

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IB antigens account for the vast majority of immune-mediated clearance.[4,5]

In a previous study, we showed that refractory patients benefit most from a transfusion from a completely HLA-matched, ABO-compatible donor. Products with some degree of HLA mismatching or ABO incompatibility may still lead to adequate corrected count increments. These mismatches are considered acceptable according to either antibody specificity, epitope matching, cross-reactive groups, the effect of previous transfusions, or any combination of these strategies.[6] In the Netherlands as in many countries, most patients who are included in the HLA-matched program have a hematologic malignancy requiring treatment with intensive chemotherapy during which adequate platelet support is needed. Our national blood supply organization offers platelet products including HLA platelets to all hospitals in the Netherlands. A large panel of donors has given permission to donate apheresis platelets and has been HLA typed. They can be requested to donate HLA-matched single-donor apheresis platelets for specific refractory patients. At present, we recruit new donors to be HLA typed from the 18- to 35-year-old whole blood and plasma donor group, without further selection.

The HLA system is encoded by the major histocompatibility gene complex, spanning a 3-Mb region on chromosome 6p21. This is the most polymorphic region of the genome, and allele frequencies differ highly among populations. This makes HLA matching a challenging task in genetically diverse populations. Within the United States, it has been shown that certain HLA-A and -B alleles are almost exclusively expressed in patients from African American or Asian origin, whereas these are hardly observed among Caucasians or North American Natives.[7] Moreover, in solid-organ transplantation as well as in hematopoietic stem cell transplantation, it has been shown that the probability of finding a sufficiently matched HLA-compatible donor is lower for patients from ethnic minorities as compared to Caucasian patients, since most registered donors are of Caucasian origin.[8,9] Therefore, a genetically diverse HLA-typed donor population is mandatory to ensure sufficient support for all patients.

We hypothesized that HLA-matched platelet support for non-Caucasian patients is hampered in a similar way as organ and stem cell transplantation. The Dutch Donor InSight study was aimed to gain insight into the characteristics and motivation of Dutch blood and plasma donors. This study showed that 2.6% of the Dutch plasma and whole blood donor population is of non-Dutch origin, whereas this was 19.8% in the general population.[10] In this study, Dutch origin was defined as both parents born in the Netherlands, although this definition is flawed from a genetic point of view. Moreover, another study revealed that almost two-thirds of our donor population lives in a geographic area with less than 5% ethnic diversity. In addition, donors living in an area with more than 40% ethnically diverse persons more often resigned as a blood donor.[11] This is likely to result in a donor population that is less well tailored to meet the needs of the 4 million first- and second-generation immigrants currently living in the Netherlands, comprising 23.6% of the total current population of 17 million people.[12]

In the current study, we evaluated our HLA-matched donor program by estimating the proportion of the nationwide patient population that can be supported by the current HLA-typed donor population. In addition, we explored the role of ethnic background of patients related to the number of available donors.

METHODS

Design and population

We performed a cohort study using a registry of clinically refractory patients for whom an HLA-matched product was ordered at the Unit of Transfusion Medicine of Sanquin, the Dutch national blood supply foundation. This registry started in 1994 and has had nationwide coverage since 2013. An HLA-matched product can be requested for patients with inadequate increments (1hCCI ≤7.5) on at least two random platelet transfusions when a role for HLA antibodies is suspected. In the current study, neonates were excluded, as thrombocytopenia of immunological origin in neonates is predominantly caused by transferred maternal antibodies. All HLA-matched products were apheresis derived, leukoreduced, stored in plasma, and irradiated before transfusion.[1]

Since the start of the registry, HLA typing techniques have improved significantly, and nowadays DNA-based typing has replaced serologic typing. We included only patients who had been HLA typed at least at low resolution for HLA-A and -B (DNA, two digits). We included both serologically and genetically typed donors. For genetic analyses, we converted serologic HLA typing into low-resolution DNA type.

Evaluation of donor population

In general, most patients who require platelet transfusions are being treated for a hematologic malignancy. Based on our experience, we assume that more than five donors per patient are required to ascertain sufficient transfusion support during chemotherapy and stem cell transplantation. We evaluated the current HLA-typed donor population in three different ways.

First, we determined the number of available donors, regardless of ABO blood group, for all patients' HLA phenotypes. For each phenotype, a donor-to-patient combination was categorized as matched if a patient and donor were HLA identical or compatible (i.e., donor with homozygous locus). In terms of Duquesnoy criteria, this comprises Grade A, BU, and B2U matches.[13] Second, we compared the prevalence of all HLA-A and -B antigens in patients and

donors.[13] Third, we compared the haplotypes of patients and donors. Therefore, haplotype frequencies were estimated with the iterative expectation-maximization algorithm, and deviation from Hardy-Weinberg proportions was tested for patients and donors. This analysis was performed using a software package (PyPop 0.7.0).[14,15]

Ethnicity

In the Netherlands, we do not register the ethnicity of our donors. Therefore, we estimated the most likely ethnicity, based on most likely estimated haplotype for patients and donors with HaploStats. HaploStats is an algorithm from the National Marrow Donor Program.[16] Based on data of a large US reference population, the prevalence of the most likely haplotype given the observed HLA-A and HLA-B antigens is given for different ethnic populations. We designated the origin of our donors as African American, Asian or Pacific Islander, Caucasian, Hispanic, or Native American, according to the HaploStats program.[17,18] The ethnicity with the highest prevalence for a certain haplotype was determined as most likely for that patient. For this analysis, we assumed that the distribution of the phenotypes of patients in our registry is representative of the phenotypes in our patient population. We estimated the ethnicity of patients with five or fewer donors and of a random sample of 100 patients with a high number ($n \geq 30$) of compatible donors and compared the number of patients of non-Caucasian origin in these groups. Due to privacy regulations, reporting unique haplotypes was not allowed.

RESULTS

Between 1994 and 2017, an HLA-matched platelet concentrate was requested for 1021 refractory patients. These patients received in total 12,350 HLA-matched transfusions, with a median of 5 (interquartile range [IQR], 2-15) and a maximum of 229 transfusions per patient. Patients were on average 54.4 years old, 65.4% were female, and the majority were treated for a malignant hematologic disease, predominantly acute leukemia (see table 1).

Available donors

In September 2017, the registry contained 19,478 HLA-typed platelet donors, who expressed 4770 unique phenotypes, with a median of 8 (IQR, 2-30) donors per phenotype. The most common phenotype among the donors was homozygous *HLA A*01; B*08*, which was expressed by 251 donors. The 1021 patients for whom HLA-matched platelets had been requested expressed 701 different HLA phenotypes, with a median of 1 (IQR, 1-2) patient per phenotype. The most common phenotype, expressed by 16 patients, was *HLA A*01, *02; B*07,*08*, for which 193 identical and 582 compatible donors were available. Each patient could be matched to a median of 83 (IQR, 18-266) identical or compatible donors, with a maximum of 807 donors per

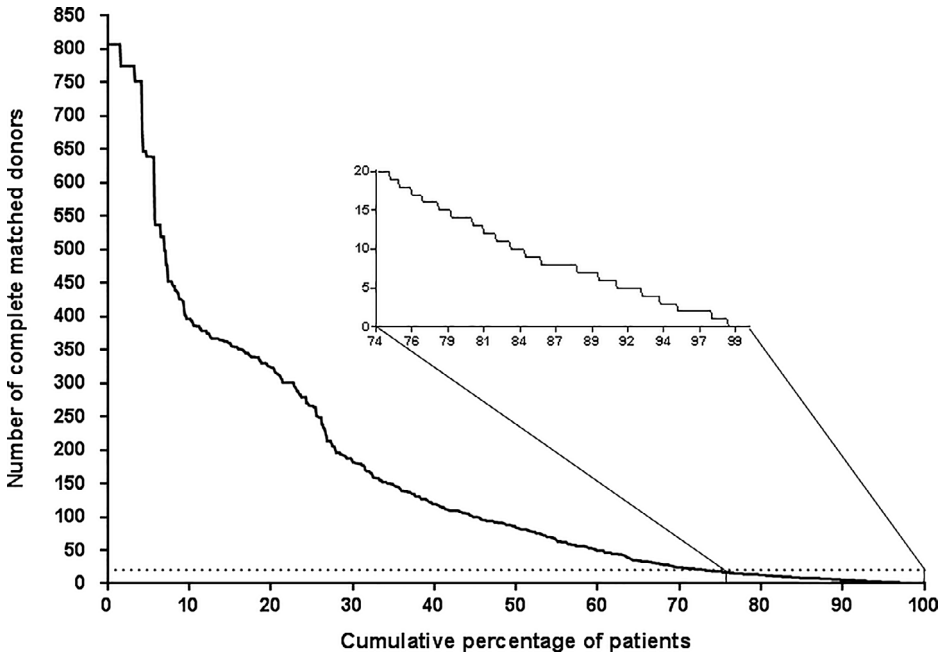

Fig. 1. Number of matched donors in the current donor population for all patients in the registry. The number of HLA identical or compatible donors per patient. The dotted line is set at 20 donors.

patient (Fig. 1). For 17 patients, all with a unique phenotype, no HLA-matched donors were available. For 95 (10.3%) patients, 5 or fewer HLA-matched donors could be found, for 161 (17.5%) patients 10 or fewer, and for 251 (27.3%) patients 20 or fewer donors were registered (Fig. 1).

TABLE 1. Demographics of the study population

Characteristics	Total registry
Patients (n)	1021
Unique phenotypes (n)	701
Female, n (%)	666 (65.4)
Diagnosis, n (%)	
Acute leukemia	404 (39.6)
Chronic leukemia	49 (4.8)
Lymphoma	51 (5.0)
Multiple myeloma	29 (2.8)
Myelodysplastic syndrome	123 (12.1)
Myelofibrosis or aplastic anemia	81 (7.9)
Benign hematologic diseases*	23 (2.3)
Solid tumor	27 (2.6)
Solid-organ transplantation	7 (0.7)
Other or unknown	166 (16.3)

* Including Glanzmann thrombasthenia, Bernard Soulier syndrome, Castelman's disease, gray platelet syndrome, thalassemia, polycythemia vera, autoimmune thrombocytopenia, immune thrombocytopenia.

TABLE 2. Hardy-Weinberg equilibrium

	p value
Patients (N=1151)	
A locus overall (common + lumped)	0.0005*
A locus homozygotes	0.0002*
A locus heterozygotes	0.1258
B locus overall (common + lumped)	0.0082*
B locus homozygotes	0.0000*
B locus heterozygotes	0.1102
Donors (N=19478)	
A locus overall (common + lumped)	0.0944
A locus homozygotes	0.0048*
A locus heterozygotes	0.1882
B locus overall (common + lumped)	0.0299*
B locus homozygotes	0.0056*
B locus heterozygotes	0.3796

* $p < 0.05$.

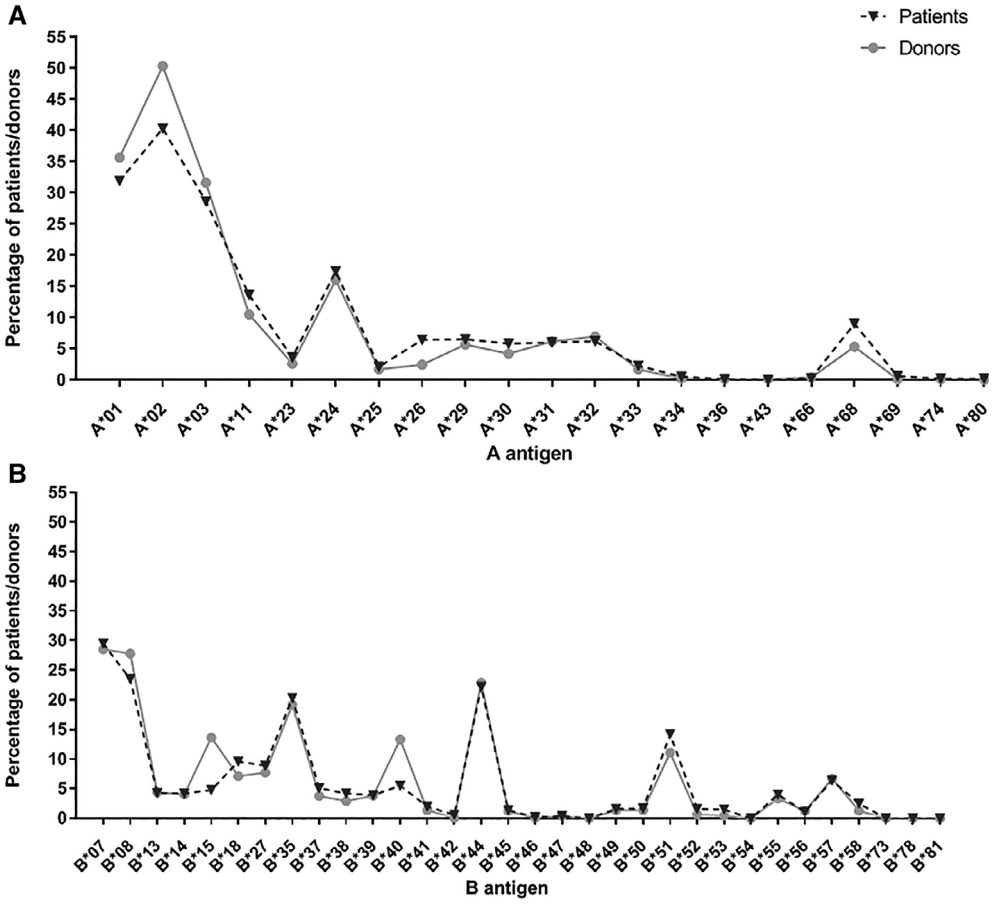

Fig. 2. Prevalence of HLA antigens among patients and donors. The percentage of donors with certain antigen is depicted with circles and the gray solid line. The prevalence among patients is reflected with triangles and a black dashed line. (A) HLA-A antigens. (B) HLA-B antigens.

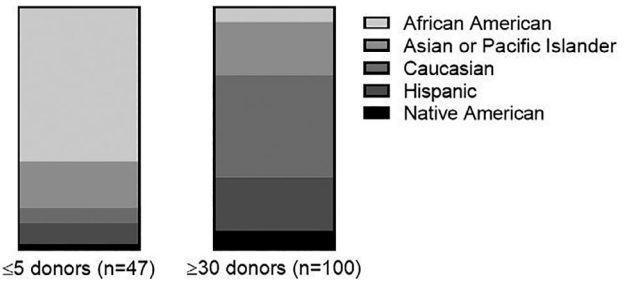

Fig. 3. Most likely population of origin for haplotypes with ≤5 donors and for haplotypes with ≥30 donors.

Evaluation of donor population

The prevalence of HLA-A and -B antigens was comparable among patients and donors (Fig. 2). The most likely haplotype among patients and donors was *HLA A*01; B*08*, which was expressed by 208 patients and 3877 donors. For 47 patient haplotypes, 5 or fewer donors were available. Fourteen unique haplotypes among patients were not expressed by any donor. In the patient cohort, genotype frequencies deviated from Hardy-Weinberg equilibrium (HWE), with an overrepresentation of homozygotes. The donor cohort showed less deviation. The overall test for the genotype frequencies of the A locus of donors is in HWE. These HWE results are summarized in Table 2.

Ethnicity

Within the donor population, 6 of the 10 most common haplotypes were most likely from Caucasian background, 2 Native American, and 2 Hispanic. Among patients, 7 of the 10 most common haplotypes were Caucasian, 2 Native American, and 1 Hispanic. Caucasian was the most likely ethnicity for 42% of patients with 30 or more donors and for 6% of patients with 5 or fewer available donors. The most likely ethnic origin for patients with 5 or fewer donors was African American (64%) and Asian Pacific (19%). In the patient group with 30 or more donors 22% were Asian Pacific and 6% were African American (Fig. 3).

DISCUSSION

Currently, almost 20,000 donors, representing 6717 unique HLA phenotypes, are available to donate HLA-matched platelets upon request in the Netherlands. In our experience, availability of more than five donors is required to ensure sufficient support during intensive treatment for hematologic malignancies. Despite nationwide coverage, insufficient numbers of completely HLA-matched donors were available for 10.3% of refractory patients. Lack of sufficient donors could hamper adequate, intensive treatment, and HLA-mismatched products result in lower increments as compared to completely HLA-matched transfusions.

The prevalence of specific HLA-A and -B antigens among these donors largely overlaps with the prevalence of these antigens among patients. All individual HLA antigens present in the patients' phenotypes are represented in the donor population as well, as we showed by simply counting the antigens. However, this is not an adequate method to evaluate the donor program, as the HLA system has strong linkage disequilibrium patterns, resulting in highly population-specific haplotypes.[19,20] Thus, an adequate evaluation can be performed only on the haplotype level.

Estimating the haplotype frequencies in the patient population revealed that there was an overrepresentation of homozygotes, causing deviation from HWE. This could not be traced back to specific antigens. In the donor population, the A locus was in HWE, whereas this was not the case for the B locus. This was also not specific for a single antigen. The deviation from HWE in the patient population could be explained by the presence of several ethnic subpopulations within this group. The donor population is in HWE, at least for the A antigen. This supports the finding that our donor population is genetically less diverse (i.e., predominantly Caucasian), whereas our patients are more representative of the admixed population in the Netherlands.

Yearly, approximately 10% of donors are no longer available to donate, creating a continuous need for a significant number of newly HLA-typed donors each year. Current practice is annual recruitment among all 18- to 35-year-old donors for HLA typing, without further selection. It is expected that in the near future the availability of low-cost genotyping platforms will vastly expand the availability of HLA-typed platelet donors. Until genotyping of all new donors becomes standard practice, it is wise to genotype donors who are expected to increase the genetic diversity of the donor population. The majority of Dutch blood and platelet donors are of Caucasian origin, whereas a relatively large proportion of patients for whom five or fewer donors were available had a non-Caucasian background, predominantly African American. The preponderance of non-Caucasian patients in the group with insufficient donors suggests that additional typing and recruitment among ethnic minorities could increase the likelihood to find a compatible donor, as it would increase the genetic variability among the donor population. Blood donorship from immigrants of mainly African ancestry could be stimulated using specific campaigns to increase awareness and to overcome differences in expectations regarding donorship.[21] Currently, we are investigating to what extent it is possible to register the ethnicity of a donor to target the typing of new platelet donors. Besides, knowledge of the red blood cell (RBC) phenotype of the current blood donor population could be used. For instance, a large proportion of Dutch blood donors is being typed for the presence of Duffy antigens Fy^a and Fy^b. The Fy^a-, Fy^b- phenotype has proven to be an accurate marker to identify donors with an African background.[22] Moreover, this method seems to be more

accurate than self-reported race.[23] Although only a small portion of the total donor population has a Fy^a–, Fy^b– phenotype, this group of donors likely add new HLA phenotypes to our current platelet donor population, in which mainly donors of African American origin are underrepresented.

Nevertheless, the HLA system is highly polymorphic. So, even with increasing genetic variability, disparities between patients and donors will sustain, and as a consequence, providing completely HLA-matched platelets to every patient with antibodies will not be feasible.[24] The benefits of additional typing of ethnic minorities would differ per population of origin. It would be especially suitable for patients from regions with limited genetic variability, mainly Asia.[25] In contrast, finding a compatible donor for an African American patient will always be more challenging, as this population has a very high degree of genetic variability compared to other populations.[8]

In conclusion, although a large donor population is available for donation of HLA-matched platelets, adequate transfusion support could not be guaranteed for 10% of refractory patients. Promoting blood donorship among residents of non-Caucasian origin and HLA typing of active blood donors with rare RBC phenotypes can be useful strategies to increase the availability of matched donors for our refractory patients.

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ALK designed research, performed research, collected data, analyzed and interpreted data, and wrote the manuscript. GWH designed research, analyzed data, and revised the manuscript. JAES collected data and revised the manuscript. BT collected data, interpreted data, and revised the manuscript. JGvdB designed research and revised the manuscript. MGJvK collected data and revised the manuscript. CMW designed research, collected data, interpreted data, and revised the manuscript.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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