

## Frequency of human platelet antigens in oncohematological patients with thrombocytopenia and the probability of incompatibility to platelet transfusions

Juliana Vieira dos Santos Bianchi<sup>1</sup>  
 Maria Regina Andrade de Azevedo<sup>2</sup>  
 Eduardo Jens<sup>1</sup>  
 Youko Nukui<sup>1</sup>  
 Dalton Alencar Ficher Chamone<sup>1</sup>

<sup>1</sup>Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo - USP, São Paulo, SP, Brazil

<sup>2</sup>Universidade Santo Amaro - UNISA, São Paulo, SP, Brazil

**Objective:** The objective of this study was to evaluate the frequencies of human platelet antigens in oncohematological patients with thrombocytopenia and to analyze the probability of their incompatibility with platelet transfusions.

**Methods:** Platelet antigen genotyping was performed by sequence-specific primer polymerase chain reaction (SSP-PCR) for the HPA-1a, HPA-1b, HPA-2a, HPA-2b, HPA-3a, HPA-3b, HPA-4a, HPA-4b, HPA-5a, HPA-5b; HPA-15a, HPA-15b alleles in 150 patients of the Hematology Service of the Hospital das Clínicas (FMUSP).

**Results:** The allele frequencies found were: HPA-1a: 0.837; HPA-1b: 0.163; HPA-2a: 0.830; HPA-2b: 0.170; HPA-3a: 0.700; HPA-3b: 0.300; HPA-4a: 1; HPA-4b: 0; HPA-5a: 0.887; HPA-5b: 0.113; HPA-15a: 0.457 and HPA-15b: 0.543.

**Conclusions:** Data from the present study showed that the A allele is more common in the population than the B allele, except for HPA-15. This suggests that patients homozygous for the B allele are more predisposed to present alloimmunization and refractoriness to platelet transfusions by immune causes. Platelet genotyping could be of great value in the diagnosis of alloimmune thrombocytopenia and to provide compatible platelet concentrates for these patients.

**Keywords:** Human platelet antigens; Platelet transfusion; Platelets

### Introduction

Human platelet antigens (HPAs) are a result of genetic polymorphisms of genes that contain the codes of amino acids of the platelet membrane glycoproteins. To date, 24 platelet antigens have been serologically defined. A single nucleotide polymorphism (SNP), characterized by the substitution of one amino acid, is present in 23 of these antigens with the exception being HPA14b. Twelve HPAs are grouped as six alleles (HPA-1 to -5 and -15), where the presence of allele antibodies occur not only for the A allele (common) but for the B allele (rare). For the other antigens, only antibodies for the B allele were detected<sup>(1-5)</sup>.

HPAs are located in receptors in the platelet membrane and are frequently involved in alloimmunization. The class I HLAs, HPA-1, -2, -3, -4, -5 and -15 respectively, are present in the GPIIIa, GPIIb, GPIIIa, GPIa and CD109 glycoproteins. According to Ghevaert et al., 95% of the antiplatelet antibodies are specific for HPA-1a or 5b; 5% of the cases involve allele antibodies for HPA-2, -3 and -15<sup>(5,6)</sup>.

The presence of antiplatelet antibodies due to an incompatibility between platelet donor and recipient or between mother and fetus during pregnancy is associated to platelet destruction and consequently thrombocytopenia resulting in severe hemorrhagic diseases such as acute neonatal thrombocytopenic purpura, neonatal alloimmune thrombocytopenic purpura, post transfusion purpura and platelet refractivity, a clinical condition in which the patient presents no increase in platelet count after blood transfusions in both non-immune and immune settings<sup>(7-11)</sup>.

Thrombocytopenia is relatively common and frequently fatal in oncohematological diseases. Prophylactic concentrated platelet transfusions occur generally when platelet counts are below 10,000/ $\mu$ L and is the main therapy to prevent bleeding. This justifies the study of HPA frequencies in oncohematological patients with thrombocytopenia as this may contribute towards an analysis of the probability of alloimmunization in transfusion therapy<sup>(12,13)</sup>.

### Methods

One hundred and fifty samples from the DNA Immuno-Hematology Laboratory of the transfusion ward of the Blood Transfusion Service of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) were selected between 1994 and 2010. Inclusion criteria were patients with oncohematological diseases and presenting with thrombocytopenia with a platelet count below 20,000 platelets/ $\mu$ L and indicated for

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#### Corresponding author:

Juliana Vieira dos Santos Bianchi  
 Hospital das Clínicas da Faculdade de Medicina, Universidade de São Paulo - USP  
 Av. Dr. Enéas de Carvalho Aguiar, 155 - Prédio dos Ambulatórios - 1º andar - bloco 7 - sala 141  
 05403-900 São Paulo, SP, Brazil  
 julianavds@hotmail.com

www.rbhh.org or www.scielo.br/rbhh

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Table 1 - Genotype and allele frequencies

	GP	NT	AA	Genotype frequency						Allele frequency	
				AA		AB		BB		A allele	B allele
				n	%	n	%	n	%		
HPA-1	GPIIIa	176T>C	Leu59Pro	107	71.3	37	24.7	6	4.0	0.837	0.163
HPA-2	GPIIba	482C>T	Thr161Met	105	70.0	39	26.0	6	4.0	0.830	0.170
HPA-3	GPIIb	2621T>G	Ile874Ser	77	51.3	56	37.3	17	11.3	0.700	0.300
HPA-4	GPIIIa	506G>A	Arg169Gln	150	100.0	0	0.0	0	0.0	1	0
HPA-5	GPIa	1600G>A	Glu534Lys	119	79.3	28	18.7	3	2.0	0.887	0.113
HPA-15	CD109	2108C>A	Tyr703Ser	37	24.7	63	42.0	50	33.3	0.457	0.543

GP = Glycoprotein; NT = nucleotide substitution; AA = amino acid substitution

concentrated platelet (CP) transfusions. The study was approved by the Ethics Committee of the Universidade Santo Amaro (UNISA - n° 043/09).

Genomic DNA was extracted from total blood samples using a DNA isolation kit (Wisard SV Genomic, Promega, EUA) according to the manufacturer's instructions and stored at -80°C. Platelet genotypes for the HPA-1a, HPA-1b; HPA-2a, HPA-2b; HPA-3a, HPA-3b; HPA-4a, HPA-4b; HPA-5a, HPA-5b; HPA-15a, HPA-15b alleles were identified using sequence-specific primer polymerase chain reaction (SSP-PCR).

The analyzed polymorphisms are located in the q21-q32 region of chromosome 17 (characterizing the HPA-1, -3 and -4 alleles), the pter-p12 region of chromosome 5 (characterizing the HPA-2 allele), the q23-q31 region of chromosome 5 (characterizing the HPA-5 allele) and the q13 region of chromosome 6 (characterizing the HPA-15 allele). Primers (Operon Biotechnologies GmbH, Colonia, Germany) were designed to flank the regions and the standard SSP-PCR technique, as described by Castro et al. and Meyer et al., was used<sup>(10,14)</sup>.

PCR reactions for the amplification of HPA-1, -2, -4, -5, and -15 were performed at a final volume of 20 µL, and for HPA-3 at a final volume of 30 µL [150 ng of genomic DNA and a mixture of 10 mM dNTP, 50 mM MgCl<sub>2</sub>, 10x buffer, 5U of Taq polymerase (Platinum, Invitrogen, Brazil) and 10mM of sense and antisense primers specific for each allele].

The reaction took place in a thermocycler (Eppendorf, Germany); the initial denaturation temperature was 95°C for 10 minutes for Taq activation, followed by 30 cycles at 95°C for 30 seconds, annealing of the primers at 65°C for 60 seconds and extension at 72°C for 30 seconds.

The genotype and allele frequencies were estimated by direct counting and the result compared to values published for healthy Brazilian individuals<sup>(15,16)</sup>.

## Statistical analysis

A conditional probability calculation was employed to estimate the probability of incompatibility. A proportional test was used to check the equivalence with other studies. A 95% confidence interval (CI) was used for the statistical analysis and for the inferential analysis a level of 5% (p-value ≤ 0.05) was considered significant with the tests being performed using a two-tailed hypothesis.

## Results

### Clinical characteristics

Of the 150 oncohematological patients with thrombocytopenia who participated in this study, 65 participants (44%) were male and 85 (56%) female. The participants' ages ranged from 13 to 80 years with a mean age of 46. According to the oncohematological pathology, 28 participants presented with aplastic anemia (18.7%), 64 were diagnosed with acute myeloid or lymphoid leukemia (42.7%), 19 participants had chronic myeloid and lymphoid leukemia (12.7%) and 39 participants were diagnosed with Hodgkin's or non-Hodgkin lymphomas (26%).

### Genotype and allele frequencies

Human platelet antigen genotypes and alleles are shown in Table 1.

The result of the A allele on HPA-1, -2, -3, -4, -5 and -15 when compared to the results reported by Medeiros<sup>(16)</sup> for HPA-1, -2, -3, -4 and -5 and by Cardone<sup>(15)</sup> for HPA-15 presented no statistically significant differences as can be seen in Table 2.

Table 2 - Comparative frequency analysis for the A allele

	Sample	Reference population	p-value
HPA-1	0.837	0.883	0.080
HPA-2	0.830	0.850	0.493
HPA-3	0.700	0.673	0.481
HPA-4	1.000	0.990	0.218
HPA-5	0.887	0.910	0.325
HPA-15	0.457	0.515	0.155

### The probability of HPA antigen incompatibility

It was necessary to analyze each genotype group (AA, AB and BB) to determine the probability of transfusion incompatibility. For participants with the AB genotype, that is with both alleles, the probability of incompatibility is either minimal or zero. The probabilities of incompatibility for participants with the AA genotype ( $IN_{AA}$ ) and for those homozygous for B ( $IN_{BB}$ ) were calculated in respect to the frequencies of donor genotypes as estimated by Medeiros<sup>(16)</sup> and Cardone<sup>(15)</sup>. The results are shown in Table 3.

Table 3 - The probability of incompatibility for AA and BB genotypes

	Donor			IN <sub>AA</sub> P <sub>AB</sub> + P <sub>BB</sub> (%)	95% CI		IN <sub>BB</sub> P <sub>AA</sub> + P <sub>AB</sub> (%)	95% CI	
	P <sub>AA</sub>	P <sub>AB</sub>	P <sub>BB</sub>		LL	UL		LL	UL
HPA-1	0.767	0.233	0.000	<b>0.233 (23)</b>	0.196	0.271	<b>1.000 (100)</b>	0.861	1.000
HPA-2	0.707	0.287	0.007	<b>0.293 (29)</b>	0.246	0.340	<b>0.993 (99)</b>	0.841	1.000
HPA-3	0.473	0.400	0.093	<b>0.493 (49)</b>	0.412	0.575	<b>0.873 (87)</b>	0.773	0.989
HPA-4	0.980	0.020	0.000	<b>0.020 (2)</b>	0.017	0.023	<b>1.000 (100)</b>	0.840	1.000
HPA-5	0.820	0.180	0.000	<b>0.180 (18)</b>	0.151	0.209	<b>1.000 (100)</b>	0.871	1.000
HPA-15	0.280	0.470	0.250	<b>0.720 (72)</b>	0.605	0.835	<b>0.750 (75)</b>	0.649	0.851

CI = confidence interval; LL = lower limit; UL = upper Limit; P<sub>AB</sub> = probability for the AB genotype; P<sub>AA</sub> = probability for the AA genotype; P<sub>BB</sub> = probability for the BB genotype; IN<sub>AA</sub> = probability of incompatibility in AA individuals; IN<sub>BB</sub> = probability of incompatibility in BB individuals

### Discussion

The study of HPA frequencies has become more relevant due to the increase in demand for platelet transfusions. Patients treated for oncohematological diseases are highly vulnerable to platelet refractivity. Bone marrow aplasia and damage to the endothelial caused by chemotherapy may result in intense and persistent thrombocytopenia and thus a need for repeated CP transfusions.

This study analyzed the HPA genotypes in patients with oncohematological diseases needing CP transfusions. The results for allele and genotype frequencies of this study were compared to the results obtained by Medeiros<sup>(16)</sup>, who determined the allele frequencies for HPA-1 to -13 using the SSP-PCR technique in 150 healthy blood donors of the Hemocentro de São Paulo (FPSHSP) and to the study of Cardone et al.<sup>(15)</sup> for the frequency of HPA-15 in 139 individuals of which 94 were blood donors.

Our results for the thrombocytopenia group presented no statistically significant differences when compared to other studies from Brazil. This shows that the A allele is more common in HPA-1, -2, -3, -4 and -5 and the B allele is more common only in HPA-15. Although Castro et al.<sup>(10)</sup>, studying Caucasian, Black and Native Indian Brazilian populations, showed that native Indians were the only group with a significant genotype difference; in the current study it was not possible to classify participants in respect to their ethnicity<sup>(10,15,16)</sup>.

The alloimmune and platelet refractivity constitute a common and relevant clinical problem that depends on such factors as type of platelet transfusion product, number of transfusions received and the immunological condition of the patient<sup>(17)</sup>. According to Ferreira<sup>(12)</sup>, approximately 20% of the platelet refractivity cases occur due to immunological causes with the development of antibodies against antigens present in the platelet membrane such as: ABO, HPA and HLA (Human Leukocyte Antigen). In spite of its clinical importance, research on antiplatelet antibodies is still incipient due to the complexity of laboratory procedures<sup>(12)</sup>.

HPA antibodies occur with a frequency of from 8 to 25% and may lead to immune refractivity. Most alloimmune and refractivity patients have anti-HPA antibodies associated to anti-HLA antibodies. Recent studies have shown alloimmunization in 8%

of the oncohematological patients that received CP transfusions with anti-HPA-5b being the most common (50%), followed by anti-HPA-1b and anti-HPA-5a, both of which are associated to platelet refractivity and both are clinically important<sup>(18-20)</sup>.

As the present study used frozen DNA samples, it was not possible to investigate anti-platelet antibodies. However, it was possible, with the analysis of genotype frequencies, to verify the probability of incompatibility of the HPA antigens.

The analysis of the probability of incompatibility of different HPAs showed that for HPA-1, -2, -4 and -5, more than 70% of individuals are homozygous for the A allele. Thus, these individuals present a probability of alloimmunization of less than 30%. Homozygous individuals for the B allele have a greater chance of alloimmunization since the probability of incompatible antigens is almost 100%.

In heterozygous individuals, the probability of alloimmunization is almost zero, since both alleles are present. The frequency and homogeneity of HPA-4 in the population reduce the probability of incompatibility to practically zero for this antigen.

Regarding HPA-3 and -15, analysis should be careful as the alleles are heterogeneous in the population<sup>(21)</sup>. In this case, homozygous individuals are at a greater risk of alloimmunization due to the greater probability of incompatibility observed in this study, in spite of the positive aspect that HPA-3 and HPA-15 appear at higher frequencies in heterozygosis (37.3% and 42%, respectively).

HPA-15 deserves special attention since it is responsible for 6.2% of all alloimmunization cases and presents limitations in the detection of antibodies due to the variation in expression of the antigen and instability of the CD109 molecule<sup>(22)</sup>.

Even though platelet refractivity is not solely due to immunological factors, it is highly probable that exposure to incompatible human platelet antigens will occur during transfusions.

Oncohematological platelet refractivity or alloimmunized patients could possibly benefit from platelet genotyping of CP donors as an additional tool to make blood transfusions compatible. Platelet genotyping associated to the use of leukocyte-reduced blood components and compatible ABO could eliminate the possibilities that result in immunologic refractivity cases.

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